

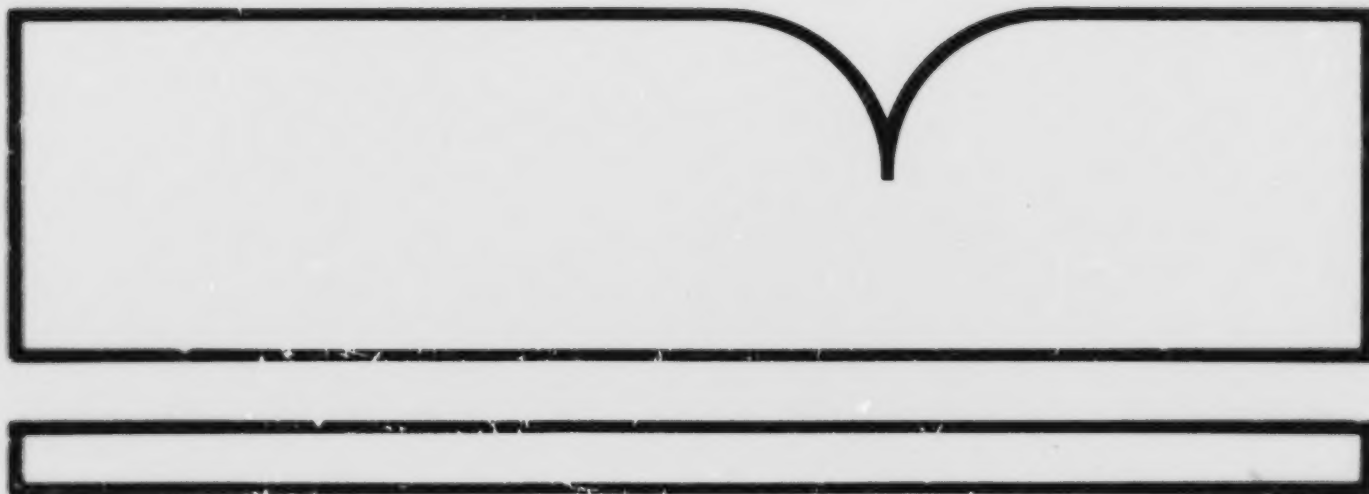
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Draft Criteria Document for
Ortho-Dichlorobenzene
Meta-Dichlorobenzene
Para-Dichlorobenzene

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(U.S.) Environmental Protection Agency
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DRAFT

DRAFT CRITERIA DOCUMENT*
FOR ORTHO-DICHLOROBENZENE,
META-DICHLOROBENZENE,
PARA-DICHLOROBENZENE

FEBRUARY 1984

HEALTH EFFECTS BRANCH
CRITERIA AND STANDARDS DIVISION
OFFICE OF DRINKING WATER
U.S. ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

*This draft criteria document contains information on three dichlorinated benzenes. At this time, an RMCL for 1,4-dichlorobenzene (p-DCB) is being proposed. Ortho- and meta- (1,2- and 1,3-) dichlorobenzene will be examined for possible inclusion in Phase II of the revised regulations.

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I. SUMMARY

There are three isomers of dichlorobenzene: ortho (1,2-), meta- (1,3-) and para- (1,4-). The major uses of ortho-dichlorobenzene (o-DCB) are as a process solvent in the manufacture of toluene diisocyanate and as an intermediate in the synthesis of dyestuffs, herbicides and degreasers (Ware and West, 1977). The bulk of para-dichlorobenzene (p-DCB) usage is in direct application as an air deodorant or insecticide which accounts for 90% of its total consumption (Lowenheim and Moran, 1975; Ware and West, 1977). The use of o- and p-DCB as deodorizers in industrial wastewaters and toilet bowl waters would suggest that increasing amounts of these substances will be found in waters throughout the country in the future. No documented uses for meta-dichlorobenzene (m-DCB) were found in the literature.

Ortho- and para- DCB are produced in considerable quantity; production volumes of o-DCB equalled 22,000 kkg and of p-DCB equalled 34,000 kkg in 1981 (USITC, 1981). Environmental releases of the dichlorobenzenes have been estimated at 30,000 kkg (or 57% of production). Approximately 7,000 kkg of o-DCB are released after solvent use and 22,000 kkg p-DCB are released from moth balls and space deodorants (SAI, 1980). Meta-DCB gets into the environment as a breakdown product of certain pesticides and as a byproduct of the manufacture of other chlorinated benzenes.

All three isomers of dichlorobenzene have been detected in drinking water supplies from both ground and surface waters, in quantities ranging from less than 0.5 ug/l to greater than 9 ug/l (EPA, 1975; EPA, 1978a).

No studies have been reported which determine the percentage of a dose of dichlorobenzene is absorbed following oral or inhalation exposure. However, for the purpose of regulation development, based upon the absorption characteristics of benzene and the smaller chlorinated ethanes and ethylenes, it will be assumed that 100% of an oral dose of any of the isomers of dichlorobenzene is absorbed and that 30% of an inhalation dose is absorbed when exposure persists for longer than one to three hours.

After oral administration to rabbits, the DCBs are oxidized principally to phenols. Ortho- and m-DCB also form catechols (Azouz, et al., 1955; Williams, 1959). The metabolites are excreted as free phenols or catechols to a slight degree, but in greater percentage as conjugates of glucuronide or sulfate. Ortho- and meta-DCB form mercapturic acids as well, but p-DCB does not (Williams, 1959). The dichlorophenols appear to be the principal metabolic products of the DCB isomers in man (Hallowell, 1959; Pagnatto and Walkley, 1965).

The ortho- and para- isomers have been shown to be quite lipophilic, and can be expected to bioaccumulate in tissues with high fat content during prolonged, continuous exposures. Para-DCB has been detected in human adipose tissue and all three isomers have been detected in blood (Dowty, et al., 1975; Morita, et al., 1975; Morita and Ohi, 1975).

Reports have appeared in the literature describing poisoning incidents resulting from exposure to the dichlorobenzenes. Girard, et al. (1969) reported four cases of leukemia in patients purportedly exposed to varying quantities and mixtures of dichloro-

benzene, although each solution contained the ortho isomer.

Hallowell (1959), Gadrat, et al. (1962), Girard, et al. (1969) and Campbell and Davidson (1970) all described cases in which individuals suffered from moderate to severe anemia following exposure to the DCBs. Several instances of skin lesions developing after contact also have been reported (Downing, 1939; Frank and Cohen, 1961; Nalbandian and Pearce, 1965).

In cases where moderate exposure to the DCBs was documented, patients complained of vomiting, headaches, irritation of the eyes and upper respiratory tract with profuse rhinitis and periorbital swelling (Dupont, 1938; Cotter, 1953; Campbell and Davidson, 1970). Anorexia, nausea, vomiting, weight loss, yellow atrophy of the liver and blood dyscrasias were reported for higher exposure concentrations (Petit and Champeix, 1954; Cotter, 1953; Wallgren, 1953; Weller and Crellin, 1953; Hallowell, 1959). Liver damage sometimes was accompanied by porphyria (Hallowell, 1959).

The dichlorobenzenes produce sedation, analgesia and anesthesia after acute oral or parenteral administration. Relatively high doses are needed to produce acute effects, but chronic effects may occur at relatively low levels. Acute poisoning is characterized by signs of disturbance of the central nervous system: hyperexcitability, restlessness, muscle spasms or tremors. The most frequent cause of death is respiratory depression. Acute exposure at high levels also may result in kidney and/or liver damage. Liver damage may be manifested as necrosis/degeneration or porphyria, depending upon the isomer to which the individual has been exposed.

The LD₅₀ for o-DCB in rats after oral administration ranged from 500 mg/kg (NIOSH, 1978) to 1,500 mg/kg (Hollingsworth, et al., 1958). In the guinea pig, the oral LD₅₀ was 2,000 mg/kg (Hollingsworth, et al., 1958). The oral LD₅₀ for p-DCB ranged from 500 mg/kg to 2,500 mg/kg (Hollingsworth, et al., 1956) in the rat and was 3,220 mg/l in the mouse (Varshavskaya, 1968). The lowest published lethal oral dose in guinea pigs was 2,800 mg/kg (Hollingsworth, et al., 1956). Irie, et al. (1973) reported an LD₅₀ of 5,145 mg/kg for a subcutaneous dose of p-DCB in the mouse.

Dogs exposed to 2 cc/m³ (0.04%) o-DCB by inhalation showed no adverse effects, whereas 0.08% produced somnolence (Riedel, 1941). Histological studies following the administration of acute and subacute doses of o-DCB showed damage to the liver and kidney. Exposing mice to the same concentrations caused CNS stimulation for about 20 minutes followed by CNS depression, muscular twitching, slow and irregular respiration, cyanosis near the end of an hour and death within 24 hours. Rats appeared to be slightly more resistant than mice to the toxic effects of o-DCB.

Inhalation of o-DCB by rats at 80 ppm for 11-50 hours was irritating to the eyes and nose, produced slight changes in the tubular epithelium of the kidney and resulted in confluent necrosis of the liver (Cameron, et al., 1937).

Rabbits, rats, and guinea pigs exposed for 20-30 minutes daily to 100 mg DCB/liter of air for 5-9 days showed marked irritation of the eyes and nose, muscle twitching, tremors, CNS depression, nystagmus and rapid but labored breathing, but recovered within 30-180 minutes after being removed from the p-DCB-rich atmosphere

(Zupko and Edwards, 1949). Body weight decreased in 11/14 rabbits and in 6/9 guinea pigs. In rats, CNS depression was observed to be greater than in rabbits. There was complete narcosis with attendant tremors and muscular twitching with each exposure. The observation that many of the test animals of all three species developed granulocytopenia is an important one. This condition is considered to be a precursor to leukemia. However, in these experiments, when the animals were removed from exposure to p-DCB, the decrease in granulocytes was reversed and the level returned to normal within three to four weeks. The question arises as to whether this condition was due to the DCB or to contamination by benzene or other substances.

Fourteen-day repeated dose gavage studies in mice and rats were conducted with both o- and p-DCB in the prechronic testing phase of the NTP bioassay on these two substances (Battelle-Columbus, 1978 a,b,d,e,f,g,h). In addition to early deaths and lack of body weight gain at the higher doses, animals exhibited histopathology indicative of hepatic centrilobular necrosis and degeneration, occasionally with cyto- and karyomegaly, as well as lymphoid depletion of the spleen and thymus.

Gavage doses of o-DCB given to rats and mice over a thirteen-week schedule of 5 days/week resulted in liver pathology indicative of necrosis and porphyria (Battelle-Columbus, 1978c, i). Serum SGPT levels were increased in mice exhibiting liver histopathology at the highest dose level. Some mice also exhibited myocardial and skeletal muscle mineralization and lymphoid depletion of the thymus and spleen and necrosis of the spleen.

Rats also showed kidney pathology as characterized by tubular degeneration.

Hollingsworth, et al. (1958) gave rats a series of 138 doses of o-DCB over a period of 192 days (18.8, 188 or 376 mg/kg/day, five days a week) by intubation. No adverse effects were detected at the lowest dose. With the intermediate dose, a slight increase in the weights of the liver and kidneys was noted. At the highest dose, there was a moderate increase in the weight of the liver, a slight decrease in the weight of the spleen and cloudy swelling of the liver.

Hollingsworth, et al. (1958) also measured the effects of multiple inhalation exposures of o-DCB on rats, guinea pigs, mice, rabbits and monkeys. A range of concentrations was used, seven hours a day, five days a week, for six to seven months. No adverse effects were observed in rats, guinea pigs or mice exposed to 49 ppm (0.29 mg/l), or in rats, guinea pigs, rabbits and monkeys exposed to 93 ppm (0.56 mg/l).

Oral doses of 10, 100 or 500 mg/kg p-DCB, five days a week, for 20 doses, produced marked cloudy swelling and necrosis in the central area of the liver nodules only with the highest dose. No effects were observed at the other doses (Hollingsworth, et al., 1956).

Thirteen week exposures by gavage to p-DCB resulted in liver pathology similar to that observed with o-DCB, but at somewhat higher doses (necrosis, degeneration and porphyria) (Battelle Columbus, 1979a,b, 1980a,b). The spleen and thymus also exhibited histopathology similar to that observed after o-DCB. In mice and

rats, hematopoietic hypoplasia of the bone marrow occurred in survivors at the highest dose (1500 mg/kg/day). Rats at the two highest dose levels also exhibited epithelial necrosis of the nasal turbinates and small intestine and villar bridging of the mucosa of the latter tissue. Again, the rats exhibited renal pathology, with multifocal degeneration or necrosis of the cortical tubular epithelium.

Oral doses of 188 or 376 mg p-DCB/kg, five days a week, for 192 days (138 doses) in rats induced an increase in the weights of the liver and kidneys (Hollingsworth, et al., 1956). At 376 mg/kg, increased splenic weight, slight cirrhosis and focal necrosis of the liver were observed. No adverse effects were seen with a 18.8 mg/kg dose.

Inhalation studies also were carried out with p-DCB (Hollingsworth, et al., 1956). The concentrations used were 96, 158, 173, 314 and 798 ppm (0.58, 0.95, 1.04, 2.05 and 4.8 mg/l, respectively). Exposure occurred for 7 hours/day, 5 days/week for 6-7 months. Adverse effects observed included liver and kidney histopathology with increased organ weights, pulmonary edema and congestion, splenic weight changes and reversible non-specific eye changes.

It is difficult to reconcile the results of the previously-described studies with those of Varshavskaya (1968). Rats received daily oral doses of 0.001, 0.01 or 0.1 mg/kg o-DCB in sunflower oil for nine months. No adverse effects were noted at the lowest dose, but varying degrees of inhibition of mitosis in the bone marrow, as well as neutropenia, abnormal conditioned

reflexes and adrenal hypertrophy occurred at the two higher dose levels.

Studies employing long-term or chronic exposures to o- and p-DCB were designed to evaluate the substances' chronic toxicity and carcinogenic potential. Preliminary assessment of the data from the NTP bioassay performed with o-DCB by gavage suggests that, under the conditions of the study, this substance is not a carcinogen in Fischer 344 rats or B6C3F1 mice (NTP, 1982). No non-neoplastic lesions were noted in either the mice or the rats, suggesting that the maximum tolerated dose was not achieved.

The results of the NTP gavage bioassay with p-DCB are not available to ODW at this time. A long-term inhalation study revealed no increase in tumor incidence or type following exposure to p-DCB in Alderly Park Wistar rats (Riley, et al., 1980a). At the high dose (500 ppm), changes indicative of non-neoplastic effects were observed: an increase in liver, kidney, heart and lung weights (both sexes) and an increase in urinary protein and coproporphyrin output (in males).

No teratogenicity studies were found in the peer-reviewed literature for any of the three isomers of dichlorobenzene. However, studies are underway to evaluate the teratogenic potential of o-DCB in rats and rabbits (Dow, 1981). In addition, results of a study by Hodge, et al. (1977) suggest that maternal exposure to atmospheric levels of p-DCB up to 500 ppm on Days 6-15 of pregnancy does not result in any embryotoxic, fetotoxic or teratogenic effects in the offspring.

Para-dichlorobenzene induces abnormal mitotic division in higher plants. Effects seen include shortening and thickening of chromosomes, precocious separation of chromatids, tetraploid cells, binucleate cells and chromosome bridges (c-mitosis) (Sharma and Battacharya, 1956; Sharma and Sarkar, 1957; Srivastava, 1966; Gupta, 1972). Ortho-DCB was shown to produce abnormal mitotic division in the onion, Allium cepa (Ostergran and Levan, 1943).

Ortho-dichlorobenzene and para-dichlorobenzene were not mutagenic when tested in a culture of histidine-requiring mutants of Salmonella typhimurium or in the E. coli WP2 system (Anderson, et al., 1972; Anderson, 1976; Simmon, et al., 1979). However, all three isomers increased the frequency of back mutation of the methionine-requiring locus in the fungus, Aspergillus nidulans (Prasad and Pramer, 1968; Prasad, 1970). In addition, the meta isomer was shown to increase mitotic recombination in the Saccharomyces cerevisiae C3 yeast system (Simmon, et al., 1979). The results with the para isomer were ambiguous. These investigators also showed that both o- and m-DCB interacted with and damaged bacterial DNA in the E. coli W3110 $polA^+/p3478\ polA^-$ differential toxicity assay system.

No evidence of mutagenicity in animals has been published to date. Guerin, et al. (1971) showed that DCB did not produce a significantly different number of mitoses in rat lung cell cultures. Cytogenetic studies with rat bone marrow cells and a dominant lethal study in CD-1 mice following exposure to p-DCB were all negative (Anderson and Richardson, 1976; Anderson and Hodge, 1976).

In the few reports available on the carcinogenic potential of the DCBs, the results are negative, although one report of four cases of leukemia in humans attributed to o-DCB or a mixture of all three isomers has been published (Girard, et al., 1969). Hollingsworth, et al. (1956, 1958) exposed several species of animals to various oral and inhalation exposures of ortho- and para-dichlorobenzene for six to seven months. No pathological changes indicative of cancerous changes were observed. In a somewhat inconclusive study, Parsons (1942) suggested that p-DCB produced a transplantable sarcoma in an irradiated mouse. Preliminary assessment of the data from the NTP carcinogenicity bioassay performed with o-DCB suggests that, under the conditions of the study, this substance is not a carcinogen in Fischer 344 rats or B6C3F1 mice (NTP, 1982). The bioassay with p-DCB has not been reported as yet. A long term inhalation study revealed no increase in tumor incidence or type following exposure to p-DCB in Alderley Park Wistar rats (Riley, et al., 1980a).

II. GENERAL INFORMATION AND PROPERTIES

Chemical and Physical Properties

Three isomers of dichlorobenzene (DCB) exist: ortho (1,2-), meta (1,3-) and para (1,4-). Very little information is available on the meta isomer. Therefore, unless otherwise noted, the properties of this isomer will be assumed to be identical to those of the ortho isomer.

At room temperature (20-25°C), ortho- and meta-DCB are colorless neutral liquids; p-DCB is a colorless crystalline solid which readily sublimates (Irish, 1963). All isomers have a molecular weight of 147.01. Each is nearly insoluble in water, but readily soluble in many organic solvents, including ethanol, benzene and diethyl ether, as well as lipid. Freed, et al. (1979) determined the solubility of p-DCB in water at 25°C to be 79 ppm (79 mg/l). All isomers are heavier than water (specific gravity = 1.306, 1.288, 1.458 at 20°C for o-, m- and p-DCB, respectively). Thus, each would tend to sink in standing water. The ortho isomer has a vapor pressure of 1.56 mm Hg at 25°C. All three isomers are combustible.

In air, $1 \text{ ppm} = 6.01 \text{ mg/m}^3$ and $1 \text{ mg/l} = 166.3 \text{ ppm}$, at 25°C and 760 mm Hg (Irish, 1963).

Sato and Nakajima (1979) determined the water/air, blood/air, olive oil/air and olive oil/water partition coefficients for o- and m-DCB. These are listed in Table II-1. With the logs of their oil/water partition coefficients approaching 4, both isomers appear to be quite lipophilic. This is confirmed by the findings of Freed, et al. (1979) who established that p-DCB has a log P (n-octanol/water) of 3.38. It would be expected that these substances would

tend to bioaccumulate in fatty tissues during prolonged, continuous exposures.

Table II-1. Partition Coefficients
(after Sato and Nakajima, 1979)

<u>Partition Coefficient</u>	<u>Ortho-DCB</u>	<u>Meta-DCB</u>
Water/Air (W)	9	5.5
Blood/Air (B)	423	201.4
Olive oil/Air (O)	39920	27080
Olive oil/Water (O/W)	4436	4924
Olive oil/Blood (O/B)	94	134
W•O	359280	148940
Log P (O/W)	3.65	3.69

The odor threshold for o-DCB in air is 2-4 ppm (AIHA, 1964). At 10-15 ppm, the smell becomes very noticeable, and at 25-30 ppm, it is considered unpleasant. Eye irritation becomes a problem at the same concentration range, while at exposures of 60-100 ppm, eye and mucous membrane irritation may be very painful. The odor threshold for p-DCB in air is 14-30 ppm in unacclimated persons (AIHA, 1964). Eye irritation begins at 50-80 ppm and becomes painful at 100 ppm. Kolle (1972) determined the odor threshold in water to range from 0.01-0.03 mg/l for the three DCB isomers.

Production and Use

o-Dichlorobenzene and p-dichlorobenzene are produced in considerable quantity. In 1981, the USITC reported that production volume for o-DCB was 22,000 kkg and for p-DCB, 34,000 kkg. At least 70 million pounds of p-DCB are used each year for moth control and as a space odorant (Brown, et al., 1975). Apparently only two U.S. companies produce m-DCB (West and Ware, 1977), and in 1974, 31,000 pounds were imported (USITC, 1974). Loss of o-DCB and p-DCB

during their manufacture amounts to at least a million pounds a year for each isomer (Brown, et al., 1975). Meta-DCB is lost as a by-product of the manufacture of monochlorobenzene. It also gets into the environment through its being a breakdown product of certain pesticides such as lindane.

The major uses of o-DCB are as a process solvent in the manufacture of toluene diisocyanate, and as an intermediate in the synthesis of dyestuffs, herbicides and degreasers (West and Ware, 1977). The bulk of p-DCB usage is in direct application as air deodorants and insecticides which account for 90% of its total consumption (Lowenheim and Moran, 1975; West and Ware, 1977). Use of o- and p-DCB as deodorizers in industrial wastewaters or in toilet bowl waters would suggest that increasing amounts of these chemicals will be found in waters throughout the country in the future. No documented uses of m-DCB were found in the literature.

III. HUMAN EXPOSURE

Humans may be exposed to dichlorobenzene in drinking water, food, and air. Detailed information concerning the occurrence of and exposure to dichlorobenzene in the environment is presented in another document entitled "Occurrence of Dichlorobenzenes in Drinking Water, Food, and Air" (Letkiewicz et al. 1983). This section summarizes the pertinent information presented in that document in order to assess the relative source contribution from drinking water, food, and air.

Exposure Estimation

This analysis is limited to drinking water, food, and air, since these media are considered to be general sources common to all individuals. Some individuals may be exposed to dichlorobenzene from sources other than the three considered here, notably in occupational settings and from the use of consumer products containing dichlorobenzene. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and several factors over which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

Unfortunately, data and methods to estimate exposure of identifiable population subgroups from all sources simultaneously have not yet been developed. To the extent possible, estimates are provided of the number of individuals exposed to each medium at various dichlorobenzene concentrations. The 70-kg male is used for estimating intake.

a. Water

Cumulative estimates of the U.S. populations exposed to various o- and p-dichlorobenzene levels in drinking water from public drinking water systems are presented in Tables IV-I and IV-II, respectively. The values in these tables were obtained using Federal Reporting Data Systems data on populations served by primary water supply systems (FRDS 1983) and the estimated number of these water systems that contain a given level of o- or p-dichlorobenzene.

Table IV-I. Total Estimated Cumulative Population (in Thousands)
Exposed to o-Dichlorobenzene in Drinking Water
Exceeding the Indicated Concentration

System type	Number of people served in U.S. (thousands)	Cumulative population (thousands) exposed to concentrations (ug/l) of:	
		<u>≥0.5</u>	>5
Groundwater	73,473	156	0
Surface water	<u>140,946</u>	<u>1,431</u>	<u>0</u>
Total	214,419	1,587	0
(% of total)	(100%)	(0.7%)	(0.0%)

Table IV-II. Total Estimated Cumulative Population (in Thousands)
Exposed to p-Dichlorobenzene in Drinking Water
Exceeding the Indicated Concentration

System type	Number of people served in U.S. (thousands)	Cumulative population (thousands) exposed to concentrations (ug/l) of:	
		<u>≥0.5</u>	>5
Groundwater	73,473	775	0
Surface water	<u>140,946</u>	<u>859</u>	<u>0</u>
Total	214,419	1,634	0
(% of total)	(100%)	(0.8%)	(0.0%)

An estimated 1,587,000 individuals (0.7% of the population of 214,419,000 using public water supplies) are exposed to levels of o-dichlorobenzene in drinking water at or above 0.5 ug/l. No individuals are estimated to be exposed to levels above 5 ug/l. Of the approximately 1.6 million people exposed to levels ranging from 0.5-5 ug/l, 1.4 million (90%) obtain water from surface water supplies.

An estimated 1,634,000 individuals (0.8% of the population using public supplies) are exposed to levels of p-dichlorobenzene in drinking water at or above 0.5 ug/l, while no individuals are estimated to be exposed to levels above 5 ug/l. Fifty-three percent of the approximately 1.6 million individuals exposed to levels ranging from 0.5-5 ug/l obtain water from surface water supplies, while 47% obtain water from groundwater supplies.

No individuals are estimated to be exposed to levels of m-dichlorobenzene above 0.5 ug/l.

No data were obtained on regional variations in the concentration of dichlorobenzene in drinking water. The highest concentrations are expected to occur near sites of production and use of dichlorobenzene.

Daily intake levels of o- and p-dichlorobenzene from drinking water were estimated using various exposure levels and the assumptions presented in Tables IV-III and IV-IV, respectively. The data suggest that the majority of the persons using public drinking water supplies would be exposed to intake levels for the dichlorobenzene isomers below 0.014 ug/kg/day.

Table IV-III. Estimated Drinking Water Intake of o-Dichlorobenzene

Exposure level (ug/l)	Persons using supplies exposed to indicated levels		Intake (ug/kg/day)
	Population	% of Total population	
≥0.5	1,587,000	0.7%	≥0.014
>5.0	0	0.0%	>0.14

Assumptions: 70-kg man, 2 liters of water/day.

Table IV-IV. Estimated Drinking Water Intake of p-Dichlorobenzene

Exposure level (ug/l)	Persons using supplies exposed to indicated levels		Intake (ug/kg/day)
	Population	% of Total population	
≥0.5	1,634,000	0.8%	≥0.014
>5.0	0	0.0%	>0.14

Assumptions: 70-kg man, 2 liters of water/day.

An indication of the overall exposure of the total population to dichlorobenzene can be obtained through the calculation of population-concentration values. These values are a summation of the individual levels of the dichlorobenzene isomers to which each member of the population is exposed. An explanation of the derivation of these values is presented in Appendix C.

Population-concentration estimates for o-dichlorobenzene in drinking water were 7.9×10^5 ug/l x persons (best case), 4.4×10^6 ug/l x persons (mean best case), 1.1×10^8 ug/l x persons (mean worst case), and 1.1×10^8 ug/l x persons (worst case). Assuming a consumption rate of 2 liters of water/day, population-exposure values of 1.6×10^6 ug/day x persons (best case), 8.8×10^6 ug/day x persons (mean best case), 2.2×10^8 ug/day x persons (mean worst case), and 2.2×10^8 ug/day x persons (worst case) were derived.

Population-concentration estimates obtained for m-dichlorobenzene were 0 (best case) and 1.1×10^8 (worst case). A median case value could not be calculated due to the absence of positive values in groundwater. Using these figures, population-exposure values of 0 ug/day x persons (best case) and 2.2×10^8 ug/day x persons (worst case) were derived.

Population-concentration estimates for p-dichlorobenzene in drinking water were 8.2×10^5 ug/l x persons (best case), 4.6×10^6 ug/l x persons (mean best case), 1.1×10^8 ug/l x persons (mean worst case), and 1.1×10^8 ug/l x persons (worst case). The population-exposure estimates derived from these values were 1.6×10^6 ug/day x persons (best case), 9.2×10^6 ug/day x persons (mean best case), 2.2×10^8 ug/day x persons (mean worst case), and 2.2×10^8 ug/day x persons (worst case).

b. Diet

Data on levels of dichlorobenzenes in foods in the United States were limited to concentrations of the chemicals in trout from the Great Lakes and in mother's milk. These data are insufficient for determining the intake of dichlorobenzene in the U.S. diet..

c. Air

Exposure to dichlorobenzene in the atmosphere varies from one location to another. The highest level of o-dichlorobenzene reported in the atmosphere

was 19,000 ng/m³ (19 ug/m³) (Bozzelli and Kebbekus 1979 cited in Brodzinsky and Singh 1982). High levels, averaging greater than 1,000 ng/m³ (1.0 ug/m³), have been detected in other areas. Normal levels, however, are somewhat lower. Brodzinsky and Singh (1982) calculated median air levels of o-dichlorobenzene for rural/remote areas, urban/suburban areas, and source dominated areas of 0.0 ng/m³ (0.0 ug/m³), 6.6 ng/m³ (0.0066 ug/m³), and 350 ng/m³ (0.35 ug/m³), respectively.

The highest level of m-dichlorobenzene reported in the atmosphere was 16,000 ng/m³ (16 ug/m³) (Wallace 1981 cited in Brodzinsky and Singh 1982). Average levels greater than 1,500 ng/m³ (1.5 ug/m³) have been reported in other areas. The following median concentrations were calculated for m-dichlorobenzene: rural/remote areas, 0.0 ng/m³ (0.0 ug/m³); urban/suburban areas, 36 ng/m³ (0.036 ug/m³); and source dominated areas, 560 ng/m³ (0.56 ug/m³).

The maximum level reported for p-dichlorobenzene in the atmosphere was 60,000 ng/m³ (60 ug/m³) (Bozzelli and Kebbekus 1979 cited in Brodzinsky and Singh 1982). Mean levels of p-dichlorobenzene above 1,000 ng/m³ (1 ug/m³) were found in two locations. Median air levels of p-dichlorobenzene for rural/remote areas, urban/suburban areas, and source dominated areas were 0.0 ng/m³ (0.0 ug/m³), 280 ng/m³ (0.28 ug/m³), and 0.0 ng/m³ (0.0 ug/m³), respectively.

The monitoring data available are not sufficient to determine regional variations in exposure levels for the dichlorobenzenes. However, urban and industrial areas generally appear to contain higher levels, as expected.

The daily respiratory intake of each of the isomers of dichlorobenzene was estimated using the assumptions presented in Tables IV-V through IV-VII and the median and maximum levels for the dichlorobenzenes reported above. The estimates in Tables IV-V and IV-VI indicate that the daily intake of o- and m-dichlorobenzene from air for adults in source dominated areas is approximately 0.1 and 0.2 ug/kg/day, respectively. A similar value (0.09 ug/kg/day) was calculated for p-dichlorobenzene in urban/suburban areas (Table IV-VII). In contrast, the intakes calculated using the maximum o-, m-, and p-dichlorobenzene levels reported are 6.3, 5.3, and 20 ug/kg/day, respectively; few if any persons are believed to be exposed at those levels. The values presented do not account for variances in individual exposure or uncertainties in the assumptions used to estimate exposure.

Table IV-V. Estimated Respiratory Intake of o-Dichlorobenzene

Exposure (ug/m ³)	Intake (ug/kg/day)
Rural/remote (0.0)	0.0
Urban/suburban (0.0066)	0.0022
Source dominated (0.35)	0.12
Maximum (19)	6.2

Assumptions: 70-kg man, 23 m³ of air inhaled/day (ICRP 1975).

Table IV-VI. Estimated Respiratory Intake of m-Dichlorobenzene

Exposure (ug/m ³)	Intake (ug/kg/day)
Rural/remote (0.0)	0.0
Urban/suburban (0.036)	0.012
Source dominated (0.56)	0.18
Maximum (16)	5.3

Assumptions: 70-kg man, 23 m³ of air inhaled/day (ICRP 1975).

Table IV-VII. Estimated Respiratory Intake of p-Dichlorobenzene

Exposure (ug/m ³)	Intake (ug/kg/day)
Rural/remote (0.0)	0.0
Source dominated (0.0) ^a	
Urban/suburban (0.28)	0.092
Maximum (60)	20

^aValue reported for source dominated areas was lower than that reported for urban/suburban areas.

Assumptions: 70-kg man, 23 m³ of air inhaled/day (ICRP 1975).

In addition to the available monitoring data, Systems Applications (1982) has provided estimates of atmospheric levels of o- and p-dichlorobenzene by applying air dispersion models to dichlorobenzene emission sources. The computed average concentrations of the dichlorobenzenes and the number of individuals estimated to be exposed to these concentrations are presented in Tables IV-VIII and IV-IX. Specific point sources in these tables are individually identified sources with known locations and modes and rates of emissions. These are generally manufacturing plants. General point sources are sources that are too numerous, small, or of uncertain location to be treated individually. However, these sources produce isolated patterns of significant concentration. Area sources are sources that are numerous and emit only small concentrations of the chemical (e.g., home chimneys, automobiles). The estimates presented for o-dichlorobenzene in Table IV-VIII suggest that only a small number of individuals (less than 700,000) are exposed to o-dichlorobenzene concentrations greater than 250 ng/m^3 (0.25 ug/m^3). Estimates for p-dichlorobenzene (Table IV-IX) suggest that 62,000,000 individuals are exposed to p-dichlorobenzene levels at or above 250 ng/m^3 (0.25 ug/m^3).

Tables IV-VIII and IV-IX also present total population-concentration estimates for o- and p-dichlorobenzene (6.45×10^6 and $5.14 \times 10^7 \text{ ug/m}^3 \times$ persons, respectively). Assuming an inhalation rate of 23 m^3 of air/day, population-exposures of 1.48×10^8 and $1.18 \times 10^9 \text{ ug/day} \times$ persons, respectively, were calculated.

SUMMARY

Tables IV-X, IV-XI, and IV-XII present a general view of the total amount of o-, m-, and p-dichlorobenzene, respectively, received by an adult male from air and drinking water. Insufficient data were obtained on levels of dichlorobenzene in foods to assess the relative intake from that source.

The data presented have been selected from an infinite number of possible combinations of concentrations for the two sources. The actual exposures encountered would represent some finite subset of this infinite series of combinations. Whether exposure occurs at any specific combination of levels is not known; nor is it possible to determine the number of persons that would be exposed to dichlorobenzene at any of the combined exposure levels. The data presented represent possible exposures based on the occurrence data and the estimated intakes.

Table IV-VIII. Exposure and Dosage Summary for Airborne o-Dichlorobenzene

Concentration level (ug/m ³)	Population exposed (persons)				Dosage (ug/m ³ x persons)		
	Specific point source	General point source	Area source	U.S. total	Specific point source	General point source	U.S. total
50	2	0	0	2	91	0	91
25	25	0	0	25	892	0	892
10	234	0	0	234	3,610	0	3,610
5	917	0	0	917	8,410	0	8,410
2.5	5,406	0	0	5,406	23,600	0	23,600
1	36,787	0	0	36,787	69,500	0	69,500
0.5	93,389	0	0	93,389	110,000	0	110,000
0.25	172,270	0	505,140	677,410	137,000	0	369,451
0.1	426,427	0	9,149,730	9,576,157	178,000	0	1,950,052
0.05	626,291	0	33,072,205	33,698,495	192,000	0	3,671,775
0.025	839,531	0	81,759,648	82,599,179	200,000	0	5,256,481
0.01	1,525,505	800	142,928,535	144,454,840	210,000	9	6,331,140
0	6,113,449	--	158,679,135	--	224,000	2,460	6,452,054

Note: The use of "--" as an entry indicates that the incremental increase in the population exposed or the dosage is not significant (relative to the last entry in that column or to an entry in another column at the same row) or that the exposure of the same population may be counted in another column.

Source: Systems Applications 1982

Table IV-IX. Exposure and Dosage Summary for Airborne p-Dichlorobenzene

Concentration level (ug/m ³)	Population exposed (persons)				Dosage (ug/m ³ x persons)		
	Specific point source	General point source	Area source	U.S. total	Specific point source	General point source	U.S. total
50	2	0	0	2	118	0	118
25	8	0	0	8	328	0	328
10	42	0	0	42	815	0	815
5	127	0	0	127	1,420	0	1,420
2.5	389	0	505,140	505,529	2,350	0	1,917,818
1	1,691	0	9,149,730	9,151,421	4,330	0	14,620,149
0.5	3,879	0	26,976,292	26,980,171	5,930	0	26,029,918
0.25	10,792	0	61,583,693	61,594,485	8,400	0	37,167,988
0.1	36,631	0	--	--	12,200	0	49,590,816
0.05	126,422	0	--	--	18,400	0	--
0.025	384,501	0	--	--	27,400	0	--
0.01	888,210	3,400	--	--	35,200	50	--
0.005	--	19,600	--	--	--	160	--
0	2,341,084	--	158,679,135	--	41,400	3,360	51,363,678
							51,408,438

Note: The use of "--" as an entry indicates that the incremental increase in the population exposed or the dosage is not significant (relative to the last entry in that column or to an entry in another column at the same row) or that the exposure of the same population may be counted in another column.

Source: Systems Applications 1982

Table IV-X. Estimated Intake of o-Dichlorobenzene
from the Environment by Adult Males in ug/kg/day
(% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air			
	Rural/remote (0.0 ug/m ³)	Urban/suburban (0.0066 ug/m ³)	Source dominated (0.35 ug/m ³)	Maximum (19 ug/m ³)
0	0.0 (--)	0.0022 (0%)	0.12 (0%)	6.2 (0%)
0.5 ^a	0.014 (100%)	0.016 (88%)	0.13 (11%)	6.2 (0.2%)
5.0 ^b	0.14 (100%)	0.14 (100%)	0.26 (54%)	6.3 (2.2%)

Intake from each source (see Sections 5.1-5.3):

Water: 0.5 ug/l: 0.014 ug/kg/day
5.0 ug/l: 0.14 ug/kg/day

Air: 0.0 ug/m³: 0.0 ug/kg/day
0.0066 ug/m³: 0.0022 ug/kg/day
0.35 ug/m³: 0.12 ug/kg/day
19 ug/m³: 6.2 ug/kg/day

Food: Not included

^a1,587,000 individuals using public drinking water systems are estimated to be exposed to levels $\geq$ 0.5 ug/l (0.7% of population using public water supplies).

^bNo individuals using public drinking water systems are estimated to be exposed to levels $>$ 5.0 ug/l.

Table IV-XI. Estimated Intake of m-Dichlorobenzene
from the Environment by Adult Males in ug/kg/day
(% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air			
	Rural/remgte (0.0 ug/m ³)	Urban/suburban (0.036 ug/m ³)	Source dominated (0.56 ug/m ³)	Maximum (16 ug/m ³)
0	0.0 (--)	0.012 (0%)	0.18 (0%)	5.3 (0%)
0.5 ^a	0.014 (100%)	0.026 (54%)	0.19 (7.4%)	5.3 (0.3%)

Intake from each source (see Sections 5.1-5.3):

Water: 0.5 ug/l: 0.014 ug/kg/day

Air: 0.0 ug/m³: 0.0 ug/kg/day
0.036 ug/m³: 0.012 ug/kg/day
0.56 ug/m³: 0.18 ug/kg/day
16 ug/m³: 5.3 ug/kg/day

Food: Not included

^aNo individuals using public drinking water systems are estimated to be exposed to levels $\geq$ 0.5 ug/l.

Table IV-XII. Estimated Intake of p-Dichlorobenzene
from the Environment by Adult Males in ug/kg/day
(% from Drinking Water)

Concentration in drinking water (ug/l)	Concentration in air		
	Rural/remote Source dominated (0.0 ug/m ³)	Urban/suburban (0.28 ug/m ³)	Maximum (19 ug/m ³)
0	0.0 (--)	0.92 (0%)	20 (0%)
0.5 ^a	0.014 (100%)	0.11 (13%)	20 (0.07%)
5.0 ^b	0.14 (100%)	0.23 (61%)	20 (0.7%)

Intake from each source (see Sections 5.1-5.3):

Water: 0.5 ug/l: 0.014 ug/kg/day
5.0 ug/l: 0.14 ug/kg/day

Air: 0.0 ug/m³: 0.0 ug/kg/day
0.28 ug/m³: 0.092 ug/kg/day
60 ug/m³: 20 ug/kg/day

Food: Not included

^a1,634,000 individuals using public drinking water systems are estimated to be exposed to levels $\geq$ 0.5 ug/l (0.8% of population using public water supplies).

^bNo individuals using public drinking water systems are estimated to be exposed to levels $>$ 5.0 ug/l.

IV. TOXICOKINETICS

Absorption/Retention

The absorption and excretion of the chlorinated benzenes take place by simple diffusion. The compounds can be absorbed from the lungs, the gastrointestinal tract and through the skin. The dichlorobenzenes are poorly soluble in water, but possess varying degrees of high lipid solubility (Neely, et al., 1974; Lu and Metcalf, 1975; Brown, et al., 1975). Thus, these substances cross most of the barrier membranes, including brain and placenta. Little information is available which demonstrates the percentage of a dose of DCB absorbed and retained following exposure in any environmental medium. Yano (1979) administered 2.5 mg p-DCB to mice orally (125 mg/kg for a 20 g mouse). Measuring carcass content of the compound (except for the gastrointestinal tract, hair, skin and tail), he determined the rate of absorption over a 24-hour period (Figure IV-1). The rate reached a maximum at 6 hours after dosing, falling to near zero after 8 hours. The author concluded that the absorption rate was $11 \pm 2.9\%$. The percentage of the dose absorbed was not identified in this study.

Based upon what is known about the absorption characteristics of benzene and the smaller chlorinated aliphatics (ethanes and ethylenes), it will be assumed that 100% of any oral dose of a dichlorobenzene is absorbed, while 30% of any DCB isomer inhaled over a period of one to several hours is absorbed and retained.

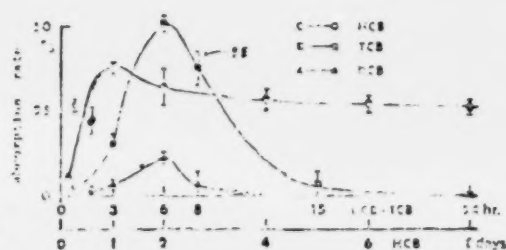


Figure IV-1. Absorption rate of DCB, TCB and HCB in mice (whole body, except for the digestive tract, hair, skin and tail) after oral administration (DCB or TCB = 2.5 mg/mouse; HCB = 1.25 mg/mouse)

Source: Yano (1979)



Distribution

Hawkins, et al. (1980) described the tissue distribution of p-DCB in adult female CFY rats following inhalation, oral or subcutaneous exposure. The animals received 10 consecutive daily exposures to p-dichloro (^{14}C) benzene either via inhalation at 1,000 ppm ($\sim 6,000 \text{ mg/m}^3$) for 3 hours/day or to a range of doses orally or subcutaneously. The compound was administered in sunflower oil at levels of 50, 125, 250, 375 or 500 mg/kg. The authors concluded that oral or subcutaneous doses of 250 mg/kg would yield tissue concentrations in fat similar to those observed after the 1,000 ppm inhalation dose.

Table IV-1 shows tissue concentrations of radioactivity in animals killed 24 hours after two or ten consecutive daily doses. During inhalation exposure, concentrations reached their maxima after six days of exposure, remaining the same or falling slightly thereafter. At the maximum point, the highest levels were found in the fat, liver and kidney. Lung and muscle concentrations reflected the levels in plasma. The highest tissue concentrations following the 250 mg/kg oral doses were reached after four doses. Again, the highest concentrations were found in liver, kidney and fat, with muscle and lung being similar to plasma. After ten doses, all concentrations had fallen somewhat, with all but fat being similar to the plasma levels.

Tissue concentrations after subcutaneous dosing at 250 mg/kg were variable, often being higher after two doses than after four or six doses, with a slight rise after six or eight doses before dropping slightly after 10. The pattern of distribution was the same as after oral or inhalation exposure.

Figure IV-2 shows concentrations of radioactivity over a 24-hour period following cessation of exposure by inhalation. Except for the lungs, concentrations were highest at 1 hour, falling thereafter. At 120 hours, the concentration in fat had fallen to about 5 ppm from 2,400 ppm at 1 hour. Concentrations in all other tissues were below the level of detection (0.2 ppm). Disappearance of radioactivity from the same six tissues was monitored in selected animals for up to 192 hours after cessation of exposure for 10 days by all three routes (Table IV-2). After oral dosing, maximum plasma concentrations occurred 2-4 hours after cessation of exposure. Peak tissue concentrations also occurred at this time, being highest in fat. Concentrations declined rapidly at all sites to undetectable levels by 120 hours. After subcutaneous dosing, peak plasma levels were reached within 1-2 hours, with peak tissue concentrations occurring at 2 hours, again highest in fat. Concentrations fell more slowly than after the other two routes of exposure, with detectable levels remaining after 192 hours.

Metabolism

Figures IV-3-IV-5 depict the metabolic pathways proposed for each of the three DCB isomers. The figures reflect a composite of work done in several laboratories.

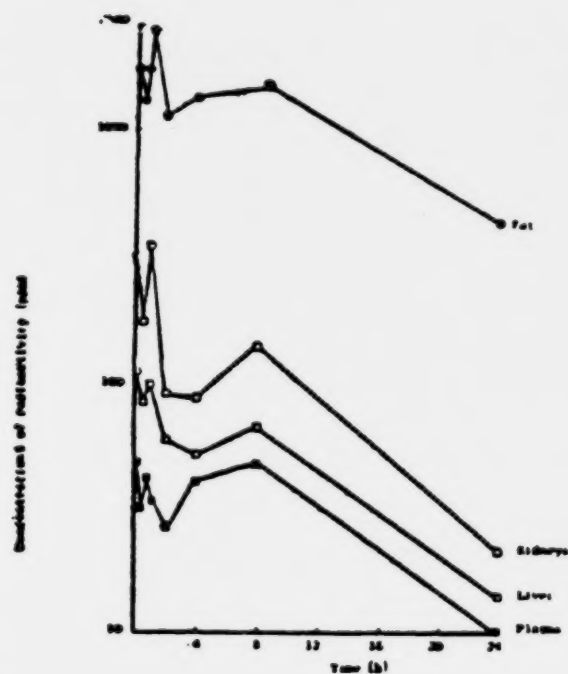


Figure IV-2. Concentrations of radioactivity in the plasma and tissues of rats after repeated daily inhalation exposures of p-dichloro (^{14}C) benzene atmospheres for ten days.

Source: Hawkins, et al. (1980)

Table IV-2

Tissue concentrations of ^{14}C in female rats at different times after consecutive daily atmospheric (inhal.) exposures (1,000 ppm) and oral or subcutaneous (s.c.) doses (250 mg/kg) of p-dichloro (^{14}C) benzene for ten days. Results are expressed as ppm and represent single animals.

Time of sacrifice (h)	Liver		Kidneys		Lungs		Muscle		Fat		Plasma							
	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.	inhal.	oral	s.c.						
0.5	83	117	23	172	74	45	178	58	24	43	12	11	1300	401	347	38	38	26
1	97	82	35	304	56	54	84	43	22	96	22	NS	2434	421	335	34	38	42
2	59	75	37	89	81	66	58	347	9	18	NS	9	1133	630	809	26	46	38
4	51	90	16	86	149	58	42	106	22	16	NS	25	1307	1423	622	40	48	29
8	66	101	30	138	123	34	39	75	18	35	23	18	1477	1385	481	48	43	26
24	14	31	28	21	31	31	9	13	18	3	11	16	425	559	476	10	18	24
48	16	7	22	15	3	38	8	3	15	5	0.2	NS	233	56	424	9	2	22
96	0.2	2	15	2	2	21	1	2	9	0.2	0.2	25	12	8	199	0.2	0.2	9
20	0.2	0.2	7	0.2	0.2	7	0.2	4	3	0.2	0.2	4	5	0.2	64	0.2	0.2	5
92	0.2	4	1	0.2	0.2	2	0.2	0.2	0.2	0.2	0.2	0.2	6	0.2	14	0.2	0.2	1

NS=No Sample

Source: Hawkins, et al. (1980)

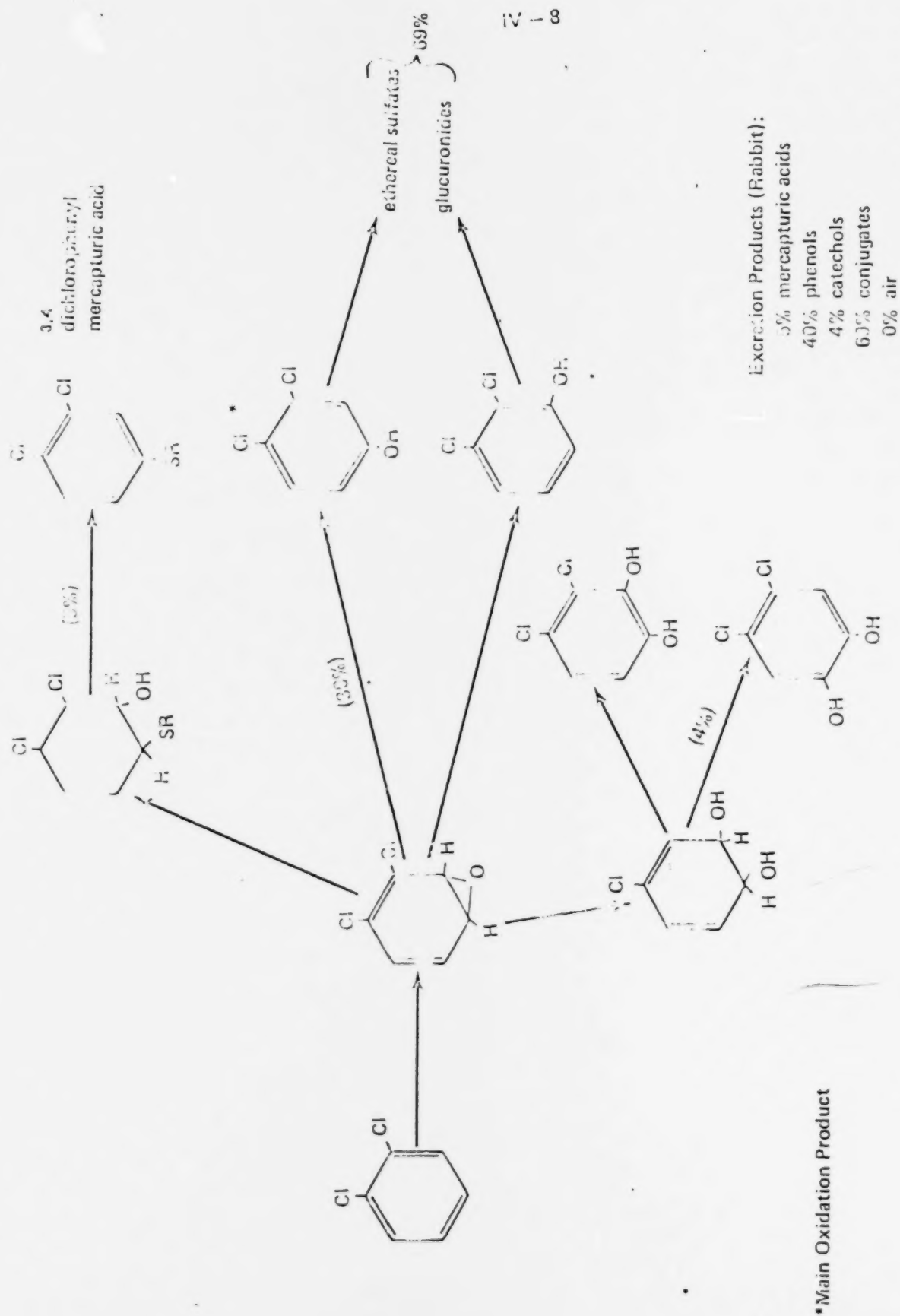


Figure IV-3. Proposed Metabolic Pathway for o-Dichlorobenzene

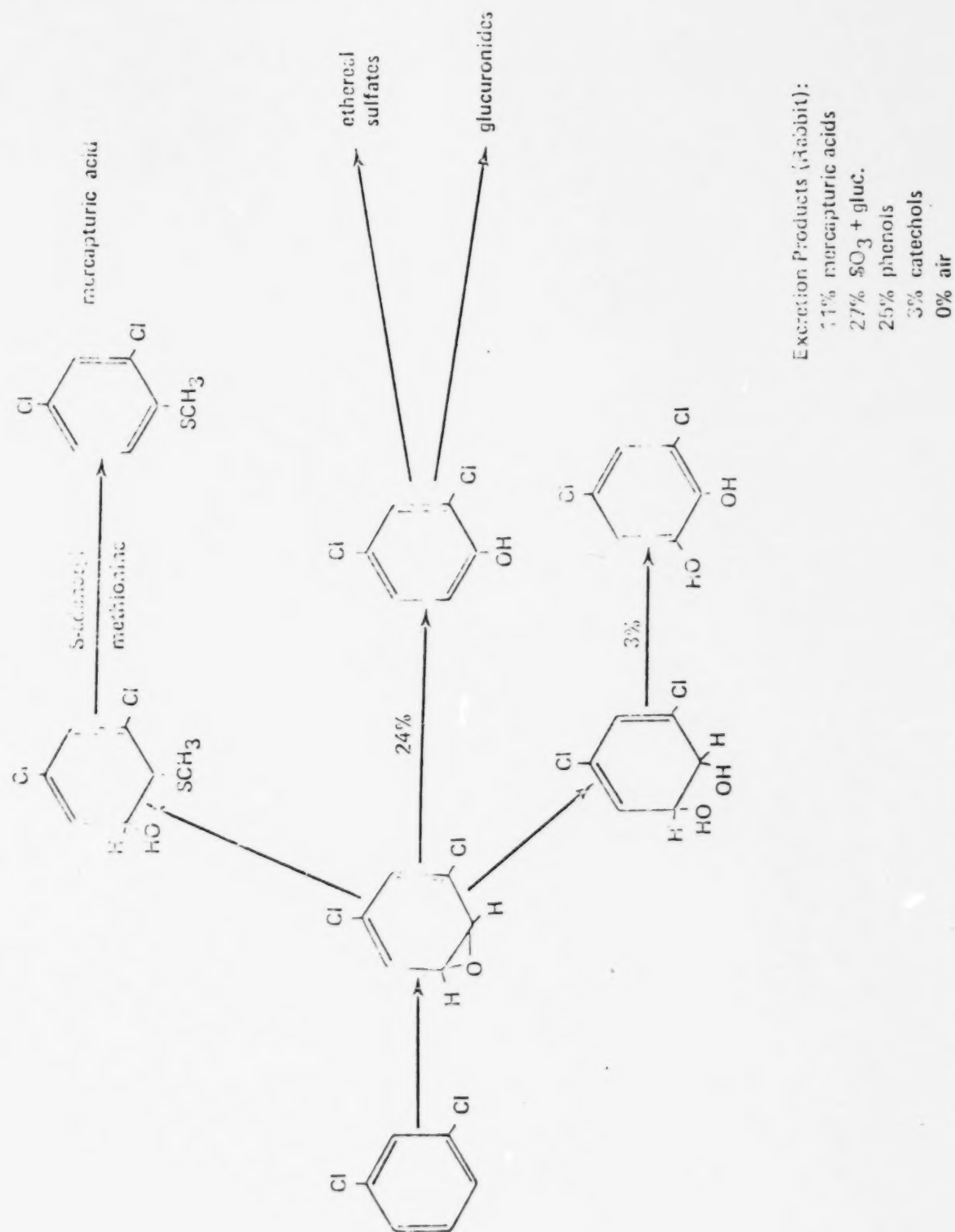
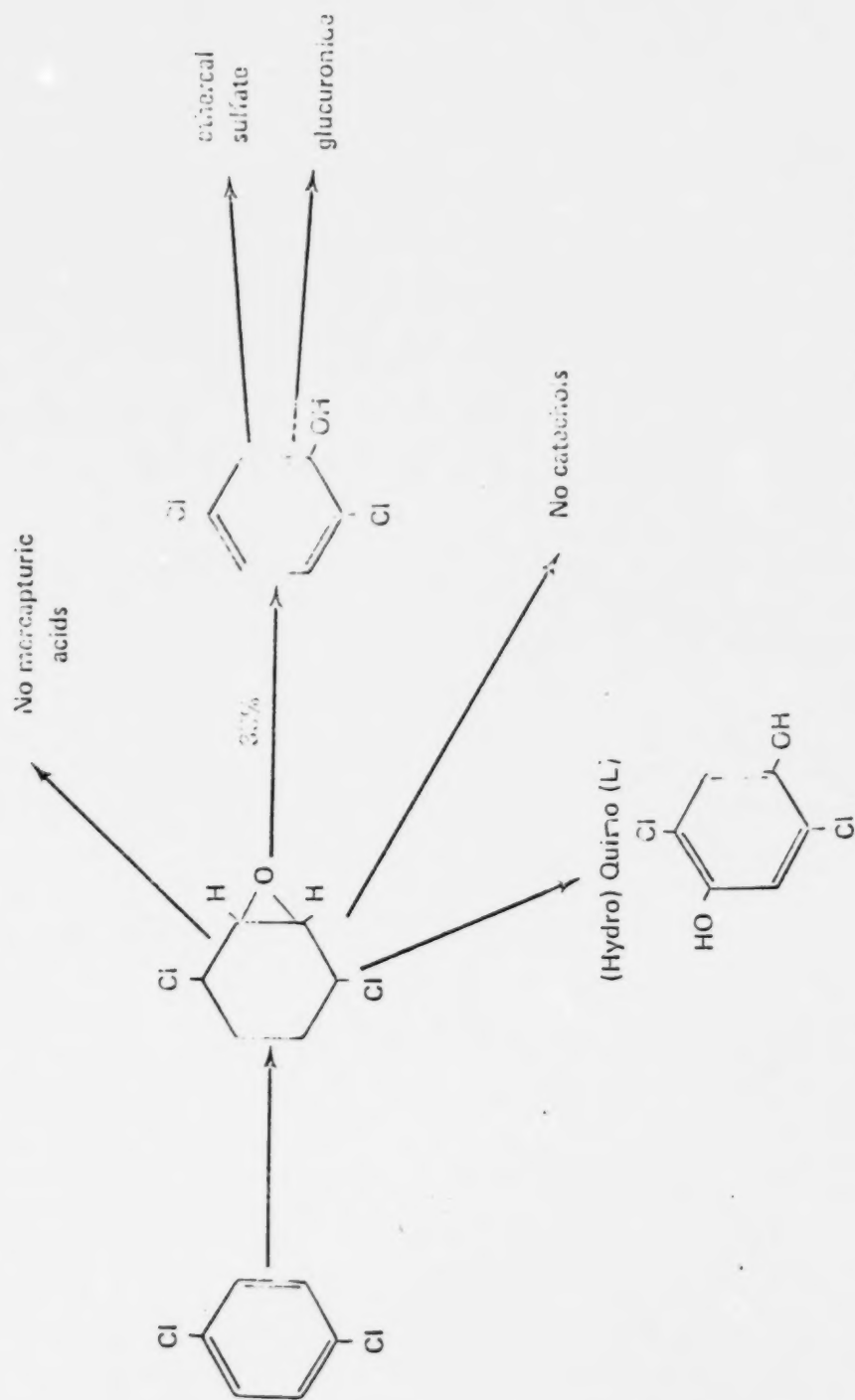


Figure IV - 4. Proposed Metabolic Pathway for m-Dichlorobenzene



Excretion Products (Rabbit):

- 0% mercapturic acids
- 55% phenols
- 6% quinols
- 65% SO_3 + gluc, conjugates
- 6% air

Figure IV - 5. Proposed Metabolic Pathway for p-Dichlorobenzene

In rabbits after oral administration (0.5 g/kg), all dichlorobenzenes were slowly metabolized by oxidation, mainly to dichlorophenols. The phenols and their conjugation products were excreted in five to six days. The major phenolic metabolite of o-DCB was 3,4-dichlorophenol (30% of the total dose), of m-DCB, 2,4-dichlorophenol (24% of the total dose) and of p-DCB, 2,5-dichlorophenol (35% of the total dose). About 3-11% of the ortho and meta isomers were excreted as dichlorocatechols. Para-DCB did not seem to form this metabolite. The ortho and meta isomers formed mercapturic acids (5-10% of the total), but p-DCB did not (Williams, 1959). The orientation of the mercapturic acid was the same as for the major phenolic product of each isomer. The phenolic metabolites were excreted as conjugates of glucuronic and sulfuric acid (Azouz, et al., 1953; Azouz, et al., 1955; Parke and Williams, 1955).

Kitamura, et al. (1977) studied the metabolism of meta- and para-dichlorobenzene in the mouse. After an intraperitoneal dose (level not stated) of either compound, urinary metabolites were characterized by GC-MS. The metabolites were the same as noted in Figures IV-4 and IV-5, with the exception that an unquantified amount of unchanged compound also was detected.

The dichlorophenols appear to be the principal metabolic product of the DCB isomers in man. In a study of industrial exposure to p-DCB, Pagnatto and Walkley (1965) used the urinary metabolite, p-dichlorophenol, as a measure of exposure to p-DCB. In a case of accidental ingestion of an unknown quantity of p-DCB crystals by a three-year old boy, analysis of urine specimens

yielded four abnormal phenols as well as 2,5-dichloroquinol. These were shown to be conjugated with glucuronic and sulfuric acids (Hallowell, 1958).

Non-mammalian animal species have been shown to metabolize the chlorinated benzenes, although the proportion of the products may differ from mammals. Safe, et al. (1976) investigated the metabolism of chlorinated benzenes in the frog, Rana pipiens. A solution of each substance studied (80 mg in 4-5 ml vegetable oil) was administered in four equal aliquots by intraperitoneal injection into each of four frogs. Para-DCB yielded trace amounts of 2,5-dichlorophenol.

Microorganisms also have been observed to metabolize the halogenated benzenes. This factor may be of some importance if the organisms inhabit the drinking water sources or waste waters which may eventually contaminate these sources.

The oxidative degradation of chlorinated benzenes by Pseudomonas was investigated by Ballschmiter and Scholz (1980). They showed that each dichlorobenzene was oxidized to one or more dichlorophenol, then to one or more dichlorocatechol. Action of the organisms on o-DCB produced primarily 3,5- and 4,5- dichlorocatechol; 3,5-dichlorocatechol was the principal product formed from p-DCB.

Garrison and Hill (1972) studied the effects of biological action on the lower chlorinated benzenes. Ortho- and p-DCB volatilized completely from aerated mixed cultures of aerobic organisms in less than one day. Midwest Research Institute (MRI, 1974) reported that p-DCB was degraded by biological organisms

to 2,5-dichlorophenol, dichloroquinol and conjugates. Gubser (1969) reported that o-DCB was degraded by sewage sludge organisms, but the degradation products were not given.

In a study with benzene-acclimated activated sludge, m-DCB was oxidized to a greater extent than either of the other two isomers after 192 hours (Malaney and McKinney, 1966). The following organisms were found in the sludge: Protozoa: Paramecium, Norticella and Episylis; bacteria: Flavobacterium lactis, Achromobacter sulfureum, A. superficialis, Alcaligenes marshallis and Rhizobium lupina. A large number of rotifers also were found during the early stages of the study.

Chlorinated Benzenes as Breakdown Products of Pesticides

Chlorinated benzenes have been identified as degradation products of lindane metabolism by various plant and animal species. Considering the once widespread use of lindane as a pesticide, some of these chlorobenzenes must have entered the environment as lindane degradation products.

Gamma-pentachloro-1-cyclohexane (-PCCH) is a known breakdown product of lindane. When corn and pea seedlings were exposed to a concentration of 25 mg -PCCH/500 ml water, both varieties converted the -PCCH to m-DCB, 1,2,4,5-tetrachlorobenzene and 2,4,5-trichlorophenol. In addition, 1,2,4-trichlorobenzene, 1,2,3,4-tetrachlorobenzene and 2,3,5-trichlorophenol were formed by the corn seedlings; 1,2,3-trichlorobenzene and 2,4,6-trichlorophenol were formed by the pea seedlings (Mostafa and Moza, 1973; Moza, et al., 1974). Gamma-PCCH, m- and p-DCB were identified in the roots of mature wheat plants that had been grown from seeds treated

treated with ^{14}C -lindane (Balba and Saha, 1974).

Mathur and Saha (1977) incubated a mineral soil and an organic soil with ^{14}C -lindane for eight weeks. While most of the lindane was recovered unchanged from the soils (70-89%), those degradation products that were identified included m- and p-DCB.

Six chlorobenzenes were "tentatively" identified as products of lindane metabolism in the rabbit (Karapally, et al., 1971). Ortho-DCB was among those detected. Chicken liver homogenates were shown to degrade lindane to all three isomers of DCB as well as some higher chlorinated benzenes (Foster and Saha, 1978). The pheasant showed a similar metabolism pattern.

Degradability and Bioaccumulation Potential

In general, the halogenated benzenes may be broken down in the environment. The extent to which they do break down depends upon a number of factors, including the type of halogen and the number and position of halogens in the ring. The lower halogenated compounds tend to be less resistant to biodegradation. The resistance of the chlorinated hydrocarbons to chemical and physical degradation and the marked ability of these compounds to accumulate in fatty tissues are the most important factors in controlling the fate and distribution of these compounds.

Both o- and p-DCB are resistant to auto-oxidation by the peroxy radical (RO_2) in water and by ozone in air (Brown, et al., 1975). The dichlorobenzenes are reactive to hydroxy radicals (OH) in air with a half-life of about three days.

Ortho- and para-DCB, in the presence of sunlight and a dilute aqueous alkali, react with chlorine to form 1,2,4-trichlorobenzene (photochlorination) (Kirk and Othmer, 1963). At moderate temperature, o-DCB is resistant to alkaline hydrolysis.

Dichlorobenzenes are decomposed by radio frequency and each DCB yields the other two isomeric DCBs (Rix and Still, 1966).

Little information is available relating to environmental hydrolysis of chlorinated benzenes. This is, of course, limited by the insolubility of these compounds in water. There is a possibility that the mono- and polyhydric phenols could be produced through hydrolysis. If environmental hydrolysis occurs, it must take place very slowly to form phenols or conjugates because of the insolubility of these compounds (Ware and West, 1977).

Alexander and Lustigman (1966) found that the presence of a chlorine atom on the benzene ring retarded the rate of biodegradation.

Para-DCB has been shown to have accumulated in human blood and adipose tissue. Morita and Ohi (1975) reported an average concentration of p-DCB in human adipose tissue of 2.3 ug/g (range = 0.2-11.7, N=34). Blood samples in six volunteers ranged from 4 ug/ml to 16 ug/ml and averaged 9.5 ug/ml. In another study, Morita, et al. (1975) reported levels of p-DCB in human adipose tissue of Tokyo residents. The concentration in the fat ranged from 0.02 ug/g to 9.90 ug/g, with a mean concentration of 1.7 ug/g. Since Morita and Ohi had established that there was a relatively high concentration of p-DCB in the atmosphere of the Tokyo metropolitan testing area (2.1-4.2 ug/m³), they believed that

inhalation of p-DCB was probably the major route of entry of the substance into the body.

In a study conducted in the New Orleans area, all three DCBs were detected in human blood samples (Dowty, et al., 1975). However, none of the chlorinated benzenes were detected in a 400 liter sample of air or in a sample of New Orleans drinking water, although many other organic compounds were confirmed. One might speculate that the individuals showing p-DCB in their blood might have been exposed to the substance during its use as a space odorant or fumigant.

Koenenmann and Leeuwen (1980) studied the kinetics of six chlorobenzenes (including p-DCB) in guppies in an accumulation and elimination experiment. The other five compounds were more highly substituted. The fish were exposed for 19 days to a mixture of the six substances, with the p-DCB concentration being 160 ng/ml in an aquarium through which the water flowed at a rate of 27 l/hr. At three-day intervals, three fish were removed from the tank and chemical analyses for the six compounds performed. Concentrations of each in ug residue/g lipid weight were determined. Average fat content of the fish was $5.4 \pm 2.0\%$.

The concentration of p-DCB in lipid reached its peak by Day 2 (150 ug/g) and remained at that level for the remainder of the exposure period. p-DCB residues were not measurable within three days after termination of exposure. The investigators calculated the log P_{oct} to be 3.53 and established the bioaccumulation factor at 1,800. Thus, while p-DCB was shown to be quite lipophilic with

preferential storage in lipid-containing tissues, it is rapidly eliminated when exposure is terminated.

Neely, et al. (1974) estimated a steady-state bioconcentration factor of 210 for p-dichlorobenzene using a short exposure and duration study with the rainbow trout.

Bioconcentration by the bluegill has been studied using ^{14}C -labeled dichlorobenzenes, with thin layer chromatography for verification (U.S. EPA, 1978b). The bioconcentration factors were 89, 66 and 60 for 1,2-, 1,3-, and 1,4-dichlorobenzene, respectively. Equilibrium occurred within 14 days and the half-life for each dichlorobenzene was less than 1 day. These results confirm that the dichlorobenzenes are unlikely to be a tissue residue problem in the aquatic environment.

V. HEALTH EFFECTS IN NON-HUMAN ORGANISMS

Plants

Para-dichlorobenzene is added to field crop seeds to control seed storage insects. It was shown that while effective in controlling pests, p-DCB significantly decreased the viability of the seeds. This effect was more pronounced when seeds were stored in a closed container rather than in an open bin (Day and Thompson, 1965). Oil seed crops, sorghum, corn and wheat were more seriously affected by p-DCB than were grasses and legumes.

Seeds were stored in Mesa, Arizona, in open and closed Mason jars (1 quart) containing 25 g p-DCB for up to eight years. No attempt was made to control temperature or humidity. After four years, none of the California Imperial flax seeds germinated from either the open or closed test container. The open control registered 77% germination after four years, while the closed control averaged 82%. Arivat barley seeds stored in the closed test jar averaged 3% germination after eight years, while germination of the barley seed in the open test jar was 54%. The control averaged 59% and 72% in the closed and open containers, respectively. After three years, none of the seed of the Pima S-1 fuzzy cotton from the closed container germinated (control = 58%). After eight years in the open test container, 43% of the fuzzy cotton seeds germinated (control = 68%).

EPA (1978b) determined 96-hour EC₅₀ values for the DCBs on a fresh water algae, Selenastrum capricornutum, and a marine algae, Skeletonema costatum, in static bioassays. In vivo

chlorophyll content and cell number counts were the parameters measured. In vivo chlorophyll content EC₅₀ values in Selenastrum were 91.6, 176 and 98.1 mg/l for o-, m- and p-DCB respectively. The EC₅₀ values for cell number count were 98 mg/l for o-DCB, 149 mg/l for m-DCB and 96.7 mg/l for p-DCB. Skeletonema was more sensitive to DCB toxicity. The EC₅₀ values for in vivo chlorophyll count were 44.2 mg/l for o-DCB, 52.8 mg/l for m-DCB and 54.8 mg/l for p-DCB. Cell number count EC₅₀ values were 44.1, 49.6 and 59.1 mg/l for o-, m- and p-DCB, respectively.

Microorganisms

The chlorinated benzenes have been shown to exhibit toxic effects upon a number of microorganisms. The antifungal vapor phase activity in the soil of each chlorobenzene was studied by Richardson (1968). A relationship between chemical structure and action was noted. Three fungal species were exposed to a range of eight vapor pressures (8-1,000 ppm). The percentage retardation of radial growth was greatest for dichloro- (all isomers) and trichlorobenzenes (both isomers).

Ortho-DCB is lethal to Mycobacterium smegmatis as a liquid or a vapor (Crowle, 1958). Torres, et al. (1970) showed that at a dilution of 1:800, o-DCB was active against Staphylococcus aureus and Escherichia coli, in vitro. In the presence of organic matter (10% defibrinated sheep blood), o-DCB was effective against the spores of Bacillus anthracis and E. coli. According to Brown, et al. (1975), p-DCB is not toxic to Ustilago maydis.

Boyles (1980) investigated the effect of ortho- and para-DCB upon the selective permeability of the cell membranes of Candida tropicalis. A culture of organisms at a concentration of 1 g/100ml was suspended in deionized water. One ml of the test compound was shaken into this suspension and conductance measured. Changes in conductance were measured across diptype bright platinum electrodes. These changes reflect leakage of electrolyte salts from the cells. The author concluded that solid hydrocarbons diffuse into cells only very slowly since ortho-DCB (which is liquid) was among the most active compounds tested (62% of internal electrolytes lost in 6 hours), whereas p-DCB (a solid) had only a very small effect (7.3% lost in six hours).

Boyles (1980) also studied the effect of a number of compounds upon the growth rate of fast growing bacteria. Among the compounds he tested was o-DCB. By employing an oxygen electrode chamber, he measured the respiration rate of the colony, suspended in a nutrient medium. Vibrio natriegens was diluted to a concentration of $\sim 10^5$ organisms/ml. Addition of increasing concentrations of o-DCB to the solution caused a dose-dependent decrease in the rate of growth. At ~ 10 ppm DCB, there was a 15% decrease. At ~ 30 ppm, the rate decreased to 20% of control and at ~ 45 ppm, the rate was reduced further to 45% of control. At ~ 60 ppm, growth was arrested completely.

Phytoplankton

Ukeles (1962) conducted a laboratory study of the tolerance of five species of marine phytoplankton to concentrations of various toxicants including o-dichlorobenzene. o-Dichlorobenzene had no

significant effect on growth of any of the tested species at 8 ppm. At 13 ppm, none of the organisms grew, but all were viable. Lethal concentrations were reached at around 80 ppm, and at 130 ppm all species were dead (See Table V-1).

Ukeles pointed out that high concentrations of toxicants used for predator control might be "safe" when used in shellfish hatcheries, though hazardous under natural conditions. This is because plankton food is grown apart from the hatchery and periodically added to it. Hence, inhibition of phytoplankton growth would not occur. In nature, phytoplankton blooms are important as a source of food. Therefore, any alteration in growth of phytoplankton resulting from use of halogenated benzenes for predator control could have consequences along the food chain.

Dawson, et al. (1977) determined the 96-hour LC₅₀ values for o-DCB in marine and freshwater fish. Tests were conducted at 23°C in five-gallon containers with aeration at pH 7.6 - 7.9. The estimated LC₅₀ value for the bluegill was 27 mg/l and for tidewater silverside, 7.3 mg/l.

EPA (1978b) determined 48-hour LC₅₀ values for the DCBs on the water flea (Daphnia magna) in static bioassays. For o-DCB, m-DCB and p-DCB, the values were 2.44, 28.1 and 11.0 mg/l, respectively. EPA (1978b) determined 96-hour LC₅₀ values on the marine mysid shrimp in biostatic assays. For o-DCB, m-DCB and p-DCB, the values were 1.97, 2.85 and 1.99 mg/l, respectively. Ninety-six hour LC₅₀ values of each DCB on the bluegill were 5.59, 5.02 and 4.28 mg/l for o-, m-, and p-DCB, respectively. In the sheepshed minnow, the 96-hour LC₅₀ values were 9.66, 7.77 and 7.40 mg/l for o-, m- and p-DCB, respectively.

Table V-1

Effects of o-DCB on Growth of Marine Phytoplankton

Concentration (ppm) of ODCB	<u>Protococcus</u> <u>sp.</u>	<u>Chlorella</u> <u>sp.</u>	<u>Dunaliella</u> <u>euchlora</u>	<u>Phaeodactylum</u> <u>tricornutum</u>	<u>Monochrysis</u> <u>lutheri</u>
1.3	0.71	0.82	0.71	0.74	1.00
7.6	0.80	0.95	0.90	0.80	0.65
13	0.00*	0.00*	0.00*	0.00*	0.00*
130	0.00	0.00	0.00	0.00	0.00
<u>Lindane</u>					
ppm					
concentration					
7.5	0.75	0.36	0.73	0.00	0.00
9	1.0	0.33	0.60	0.00*	0.00

* no growth, but organisms viable

All numbers represent the ratio of optical density (o.d) of growth in the presence of toxicants to o.d. in the basal medium with no added toxicants. Hence 1 = approximately normal growth.

Source: Ukeles, 1962.

In a 30-day continuous flow chronic toxicity study, EPA (1978b) determined the maximum acceptable toxicant concentration (MATC) for o-DCB toward the fathead minnow to be greater than 4, but less than 8 mg/l with a geometric mean of 5.65 mg/l. The "no-effect" level was then said to be 4.0 mg/l.

Several chlorinated and fluorinated benzenes are known to be toxic to a variety of marine organisms including molluscs and crustacean species. Because of this known toxicity, there have been suggestions that mixtures of these substances be spread around oyster beds to safeguard the crop from predators. Ortho-DCB, in unknown concentration, was placed in the area of an oyster bed (Loosanoff, et al., 1960). The oyster drills would not cross the barrier of o-DCB and sand eight feet wide during a 13-month period. The drills exhibited extreme swelling in the gastropods, immobility and death, and curling of the tips of their rays. Crabs coming into contact with the substance lost their equilibrium and went into convulsions. In a study of the effects of o-DCB upon clam young (Mercenaris mercenaris), Davis and Hidu (1969) found a TL_m value of 100 ppm in eggs exposed for 48 hours or larvae exposed for 14 days. Oysters, themselves, are not immune from the toxic effects of o-DCB. An exposure of 1 ppm was found to be the minimum concentration which inhibited the growth of young oysters (Crassosterea virginia) after 24 hours' exposure (Butler, et al., 1960). No bioaccumulation was noted; the DCB was excreted by the oyster when the chemical was removed from the water.

Insects

Both o- and p-DCB have been used as insecticides; of the two, p-DCB has received more widespread application. However, only a few toxicity studies are available for these substances.

The acute effects of insect exposure to chlorinated benzenes are summarized in Table V-2.

Birds

The acute and subacute toxicity of p-DCB was studied by Hollingsworth, et al. (1956). Pekin ducks were fed a diet of 0.5% p-DCB for 35 days. Growth was retarded in all animals, and three of the animals died.

Non-human Mammals

The acute and chronic toxicities of the dichlorobenzenes closely parallel those observed with chlorobenzene. A search of the literature uncovered no data on the acute or chronic toxic effects on m-DCB. However, a number of studies have been published on the acute and long-term effects of the other two dichlorobenzene isomers. In general, p-DCB found to be less toxic than the ortho-isomer.

The dichlorobenzenes produce sedation, analgesia and anesthesia after acute or parenteral administration. Relatively high doses are needed to produce acute effects, but chronic effects may occur at relatively low levels. Acute poisoning is characterized by signs of disturbance of the central nervous system (CNS). There may be hyperexcitability, restlessness, muscle spasms or tremors followed by varying degrees of CNS depression. The most

Table V-2

Acute Effects of Chlorobenzenes in Insects (West and Ware, 1977)

<u>Chemical</u>	<u>Organism</u>	<u>Exposure</u>	<u>Remarks</u>	<u>Reference</u>
o-DCB	<u>Cimex lectur</u> <u>is</u>	Fumigant	Nervous System effects	Cameron, <u>et al.</u> , 1937
o-DCB	<u>Dendroctonus pseudotsagae</u>	1 part o-DCB 5 parts diesel oil	100% mortality	Gibson, 1957
p-DCB	<u>Calandra granaria</u>	Fumigant	Nervous System effects	Cameron, <u>et al.</u> , 1937
p-DCB	<u>Aphis rumicis</u>		Narcosis	Tatterfield, <u>et al.</u> , 1925
p-DCB	<u>Periplaneta americana</u>	Injection	Nervous system effects-increase in activity followed by a decrease and spasmodic contractions	Munson and Yaeger 1945
p-DCB	<u>Carabus memoralis</u> <u>Calliphora erthnocephala</u> <u>Triatoma rubro-fasciata</u>	Vapor	Respiratory and nervous system stimulated, then depressed. Delayed increase in CO ₂ output at 4 hours, then a decrease	Punt, 1950
p-DCB	<u>Cambarus virilis</u>	Allied to nerve sheath	Facilitation, then depression of transmission	Punt, 1950

Table V-2 (Continued)

<u>Chemical</u>	<u>Organism</u>	<u>Exposure</u>	<u>Remarks</u>	<u>Reference</u>
p-DCB	Larvae of: <u>Tineola</u> <u>bisselliella</u>	Vapor	100% mortality	Batth, 1971
	<u>Attagenus megatoma</u>			
p-DCB	<u>Attagenus piceus</u>	2.3-3.2 mg/l	Low concentration repelled larvae; high concentration killed 100% after 4 days	Arnold, 1957
p-DCB	Termites: <u>Glyptotermes dilatatus</u> <u>Kaloterme</u>	solid or liquid fumigant (with TCBS)	Effective in control	Dantharayana and Fernando, 1970a, 1970b
p-DCB	<u>Lyctus africanus</u>	25% solution	100% mortality within 8 days	Awad, 1971
p-DCB	<u>Tyrophagus dimidiatus</u>	0.5% solution v/v	100% mortality within 4 minutes	Honma, 1967

frequent cause of death is respiratory depression. Acute exposure at very high levels also may result in liver or kidney damage. Certain of the halogenated benzenes, like some aliphatic hydrocarbons, are thought to sensitize the myocardium to the effect of catecholamines, and thus set up conditions favoring ventricular arrhythmias (von Oettingen, 1955).

Acute Exposure

Several investigators have determined the acute lethal dose levels after exposure to the dichlorobenzenes in several species. These data are presented in Table V-3.

Varshavskaya (1968), in her comparative studies on the adverse effects of the lower chlorinated benzenes, showed that, in the acute exposures, o-DCB was slightly less toxic in mice and rats than monochlorobenzene (MCB), and that p-DCB was even less toxic than o-DCB or MCB. o-DCB was slightly more toxic than MCB in rabbits and guinea pigs. The LD₅₀ values for acute oral doses of o-DCB were: 2,000 mg/l for the mouse, 2,138 mg/l for the rat, 1,875 mg/l for the rabbit and 3,375 mg/l for the guinea pig. The LD₅₀s for p-DCB were found to be 3,220 mg/l for the mouse, 2,512 mg/l for the rat, 2,812 mg/l for the rabbit and 7,593 mg/l for the guinea pig.

Doses of 0.25-0.5 cc/kg (0.33-0.66 mg/kg) body weight of o-DCB intravenously administered to rabbits were fatal within 24 hours; doses of 1.0 cc/kg (1.31 mg/kg) were fatal within 20 seconds (Cameron, et al., 1937).

Dogs exposed to 2 cc/m³ (0.04% or ~400 g/m³) o-DCB via inhalation showed no adverse effects, whereas 0.08% (~800 g/m³) produced somnolence (Riedel, 1941). Histological studies

Table V-3

Acute Toxicity Data for o- and p-Dichlorobenzene

<u>Animal</u>	<u>Route</u>	<u>LD₅₀</u>	<u>LCL₀</u>	<u>Reference</u>
<u>o-Dichlorobenzene</u>				
Rat	Oral	500 mg/kg		NIOSH, 1978
Rat	Oral	2138 mg/l		Varshavskaya, 1968
Mouse	Oral	2000 mg/l		Varshavskaya, 1968
Rabbit	Oral	1875 mg/l		Varshavskaya, 1968
Guinea Pig	Oral	3375 mg/l		Varshavskaya, 1968
Guinea Pig	Oral	2000 mg/kg		Hollingsworth, et al., 1958
Rat	Inhal.		821 ppm/7 hr	Hollingsworth, 1958
Guinea Pig	Inhal.		800 ppm/7 hr	Hollingsworth, 1958
Guinea Pig	Inhal.		800 ppm/24 hr	Cameron, et al., 1937
<u>p-Dichlorobenzene</u>				
Rat	Oral	500 mg/kg		NIOSH, 1978
Rat	Oral	2500 mg/kg		Hollingsworth, et al., 1956
Rat	Oral	2138 mg/l		Varshavskaya, 1968
Mouse	Oral	3220 mg/l		Varshavskaya, 1968
Rabbit	Oral	2812 mg/l		Varshavskaya, 1968
Guinea Pig	Oral	7593 mg/l		Varshavskaya, 1968
Guinea Pig	Oral	2800 mg/kg	(LDL ₀)	Hollingsworth, et al., 1956
Mouse	SC	5145 mg/kg		Irie, et al., 1973

following the administration of acute and subacute doses of o-DCB showed damage to the liver and kidneys. Exposing mice to similar concentrations caused CNS stimulation for about 20 minutes followed by CNS depression, muscular twitching, slow and irregular respiration, cyanosis near the end of an hour, and death within 24 hours. Rats appeared to be slightly more resistant than mice to the toxic effects of o-DCB.

In the mouse, the LD₅₀ value for a subcutaneous dose of p-DCB was found to be 5,145 mg/kg (Irie, et al., 1973).

Inhalation of o-DCB by rats at 800 ppm (4,800 mg/m³) concentration for 11-50 hours was irritating to the eyes and nose, produced slight changes in the tubular epithelium of the kidney and resulted in confluent necrosis of the liver (Cameron, et al., 1937).

Rabbits, rats and guinea pigs exposed for 20-30 minutes daily to 100+ g p-DCB/m³ of air for 5-9 days showed marked irritation of the eyes and nose, muscle twitching, tremors, CNS depression, nystagmus and rapid but labored breathing (Zupko and Edwards, 1949). The animals recovered within 30-180 minutes after being removed from the p-DCB-rich atmosphere. A definite granulocytopenia was observed in 11 rabbits, a questionable change in three others and no change in the remaining three. Body weight decreased in 14 animals; three rabbits showed an increase. In 13 rats, CNS depression was observed to be greater than in rabbits. There was complete narcosis with attendant tremors and muscle twitching with each exposure. A definite granulocytopenia was observed in eight rats, a questionable change in three, and no change in two others. Nine rats showed a decrease in total white cell count

while four showed an increase. In addition to exhibiting similar symptoms to p-DCB inhalation as rats and rabbits, guinea pigs also exhibited granulocytopenia in most cases. Five animals showed a frank decrease in granulocytes, two showed a tendency toward lowering, while two others showed no decrease. Six of the nine animals suffered a weight loss while three did not.

The observation that many of the test animals developed granulocytopenia is an important one. This condition is considered to be a precursor to leukemia. However, in these experiments, when the animals were removed from exposure to p-DCB, the decrease in granulocytes was reversed and the level returned to normal within three to four weeks.

Several animal studies document the effects of o- and p-DCB on the eye and/or skin. The results are summarized in Table V-4.

Yang, et al. (1979) showed that both o- and p-DCB alter pancreatic function. They administered a single intraperitoneal dose of each substance in sesame oil (5 mmol/kg: 735 mg/kg) to fasted adult male Holtzman rats. Control animals received an equivalent volume of sesame oil (1 ml/kg). Experimental measurements were made 24 hours later. After treatment with o-DCB, bile duct-pancreatic flow (BDPF) was increased nearly ten-fold over that observed in control animals ($P < 0.05$). p-DCB did not alter the flow significantly. Protein concentration of the bile effluent was reduced to about 25% after o-DCB ($P < 0.05$), but was not changed after p-DCB. However, p-DCB did increase significantly the chloride content of the effluent, whereas o-DCB did not. Neither compound affected the rate of bile flow or serum levels of SGPT. The authors

Table V-4

Effects of Chlorinated Benzenes on the Eye and Skin
(modified from West and Ware, 1977)

Chemical	Animal	Exposure	Effects	Reference
o-Dichloro- benzene	Rabbits	2 drops in each eye	Pain slight. Conjunctival irritation. Cleared in 7 days	Hollingsworth, et al., 1958
p-Dichloro- benzene	Rabbits	4.6 to 4.8 mg/l (770-800 ppm) for 8 hours, inhalation	Lateral nystagmus, transitory edema of cornea. - edema of optic nerve. Eye changes reversible (17 days). No lens changes or deposits in the vitreous.	Pike, 1944
p-Dichloro- benzene	Rabbits	Inhalation. 5 gm vaporized/2 days 5-47 days	No opacity of the lens (liver necrosis and death)	Berliner, 1939
p-Dichloro- benzene	Rabbits	Oral, 5 gms p-DCB daily for 3 weeks	Opacity of lens slight and mod	Berliner, 1939

could not offer an explanation of the mechanism by which the observed changes occurred, although they did conclude that the mechanism did not involve secretin or cholinergic stimulation of the pancreas. Thus, the significance of these findings remains unknown.

Effects on Porphyrin Metabolism

All of the chlorinated benzenes have been shown to produce disturbances in porphyrin metabolism. The mechanism for this biochemical lesion seems similar to that seen following administration of other inducers of drug metabolism. It is also similar to that seen in man following exposure to ethanol, synthetic estrogens and progestins (Parke, 1972).

Rimington and Ziegler (1963) showed that in male albino rats administration by gavage of 500 to 1,200 mg/kg/day for 5-15 days of chlorinated benzenes (except pentachlorobenzene) leads to a hepatic porphyria characterized by elevated levels of precursor porphyrins in liver and feces. The investigators administered o-DCB in liquid paraffin at maximum dose levels of 455 mg/kg/day for 15 days, and p-DCB, also in liquid paraffin, at maximum dose levels of 770 mg/kg/day for 5 days. Doses were gradually increased until a level was reached which yielded high porphyrin excretion, but few fatalities. The first signs of intoxication were an increase in urinary coproporphyrin and porphobilinogen (PBG). An increase in aminolevulinic acid excretion was a late effect. The most potent compound was p-dichlorobenzene (see Table V-5). Mean peak values of urinary coproporphyrin increased 10-15 fold after p-DCB to about 62 ug/day when compared with control. o-DCB treatment elicited a 6-10 fold increase in the same parameter (to about 43 ug/day). After p-DCB,

Table V-5

Mean Peak Values of Urinary Porphyrin Precursors Following Treatment
With Maximum Doses Tested for each Chlorinated Benzene
(Rimington and Ziegler, 1963)

No. of rats	Compound	Sol- vent	Max. dose (mg/kg)	Days on max. dose	Copro- porphyrin (pg/day)	Uropor- phyrin (pg/day)	PBG (pg/day)	ALA (pg/day)	ALA/PBG
	Controls*	C			4.3-6.8	0.1-0.3	2.5-6.5	38.7-51.6	
3	Monochlorobenzene	P	1140	5	30.50	1.40	26.70	56.40	2.98
3	1,2-Dichlorobenzene	P	455	15	43.10	2.01	26.65	11.32	2.11
3	1,4-Dichlorobenzene	P	770	5	61.80	10.99	1328.10	437.41	0.36
3	1,2,3-Trichlorobenzene	C	785	7	36.59	0.72	57.38	36.26	1.01
3	1,2,4-Trichlorobenzene	C	730	15	58.31	2.73	179.00	145.70	1.06
3	1,2,3,4-Tetrachloro- benzene	P	660	10	28.96	3.80	520.63	315.78	0.61
6	1,2,4,5-Tetrachloro- benzene	C	905	5	4.10	0.22	6.50	18.08	6.69

P = liquid paraffin; C = 1% cellosol

PBG = porphobilinogen; ALA = γ -aminolevulinic acid.

* Mean max. and min. of 5 rats during 41 days.

a nearly 100-fold increase in urinary uroporphyrin levels occurred, while o-DCB caused an increase of about 10-fold. Porphobilinogen levels measured 1,328 ug/day after p-DCB, but only 26.65 ug/day after o-DCB, increasing from a control level of 2.5-6.5 ug/day. After p-DCB, a 10-fold increase (to 437 ug/day) in δ -ALA levels were observed, while after o-DCB, levels actually decreased to 11.3 ug/day (normal range = 38.7-51.6 ug/day.)

Several parameters of liver function also were measured by Rimington and Ziegler (Table V-6). In contrast to the change observed in urinary coproporphyrin excretion, liver coproporphyrin was unchanged after p-DCB, and roughly doubled after o-DCB (to 10.05 ug/100g tissue vs. 4.5 ug/100g in the controls). Protoporphyrin levels increased six-fold after p-DCB (60.5 ug/100g), but only 3.5-fold after o-DCB (to 34.8 ug/100g from a control level of 9.7 ug/100g). Catalase levels were unaffected by p-DCB, but more than halved after o-DCB (0.85 meq/mg wet weight for control to 0.364 meq/mg wet weight after p-DCB exposure). These decreases in catalase were observed only in animals in which marked histological changes indicative of severe liver damage with large areas of necrosis were observed. Thus, changes occurred in o-DCB-treated animals, but not in those treated with p-DCB. In addition, p-DCB did not alter the glutathione content of the liver. o-DCB was not tested. From these data, the authors suggested that the mechanisms which produce porphyria derangements may be different from those which lead to liver necrosis.

Carlson (1977) studied chlorinated benzene induction of liver porphyria in groups of five female rats receiving p-DCB,

Table V-6

Porphyrins, Porphobilinogen and Catalase Activity
In Livers of Rats Treated With Chlorinated Benzenes
(Rimington and Ziegler, 1963)

No. of rats	Compound	Max. dose (mg/kg)	Days on max. dose	Copro- porphyrin (ug/100 day)	Protopor- phyrin (ug/100 g)	Uropor- phyrin (ug/100 g uncorrected)	PPG	Catalase (meg/mg) wet wt.
2	Controls			4.5	9.7	1.3	—	0.85
2	Monochlorobenzene	1400	5	Trace	13.00	6.35	—	0.502
2	1,2-Dichlorobenzene	450	15	10.05	34.80	14.40	—	0.364
2	1,4-Dichlorobenzene	770	5	5.07	60.35	60.35	—	0.880
2	1,2,3-Trichlorobenzene	780	7	2.65	3.55	20.00	—	0.857
5	1,2,4-Trichlorobenzene	500	10	45.56	55.60	52.70	++	0.747
3	1,2,3,4-Tetrachloro- benzene	660	10	35.04	56.57	41.32	+	0.772
2	1,2,4,5-Tetrachloro- benzene	850	5	6.30	9.90	2.15	—	0.838

1,2,4-trichlorobenzene or hexachlorobenzene. Each substance was suspended in corn oil and administered orally at 0, 50, 100 or 200 mg/kg/day for 30, 60, 90 or 120 days. After the last dose, the animals were placed in metabolism cages for collection of 24-hour urine samples. At the end of the 24-hour period, the animals were sacrificed and liver and urinary porphyrins measured. The results after exposure to p-DCB can be seen in Table V-7. The data show that this substance has little potential for causing porphyria, thus confirming the observations of Rimington and Ziegler (1963). After 30 and 60 days, liver weight increased in a dose-dependent manner. Even after 120 days, only slight increases in liver porphyrins occurred. Urinary excretion of -ALA, porphobilinogen (PBG) or porphyrins was not increased over control levels.

Subacute to Longer-term Exposures

A number of reports have appeared in the literature that describe subacute to longer-term exposures of experimental animals to the ortho- and para- isomers of dichlorobenzene (see Table V-8 for a summary of these data).

Ortho-Dichlorobenzene

Hollingsworth, et al. (1958) gave rats a series of 138 doses of o-DCB over a period of 192 days (18.8, 188 or 376 mg/kg/day, five days a week) by intubation. No adverse effects were detected at the lowest dose. With the intermediate dose, a slight increase in the weight of the liver and kidneys was noted. At the highest dose, there was a moderate increase in the weight of the liver, a slight decrease in the weight of the spleen and cloudy swelling of the liver.

Table V-8

Effect of 1,4-Dichlorobenzene p.o. on Porphyrin Production
and Excretion in Female Rats.

(Carlson, 1977)

Dose (mg/kg)	Liver wt(g)	Liver porphyrins (ng/g)	Urine porphyrins (ug/24h)
30 days of administration			
0	6.8 $\pm$ 0.3 ^a	246 $\pm$ 21 ^a	1.5 $\pm$ 0.2 ^a
50	6.6 $\pm$ 0.4 ^a	269 $\pm$ 20 ^a	1.9 $\pm$ 0.3 ^a
100	7.0 $\pm$ 0.2 ^a	251 $\pm$ 22 ^a	1.4 $\pm$ 0.2 ^a
200	8.0 $\pm$ 0.3 ^b	276 $\pm$ 18 ^a	1.4 $\pm$ 0.2 ^a
60 days of administration			
0	6.7 $\pm$ 0.3 ^a	381 $\pm$ 20 ^a	1.9 $\pm$ 0.4 ^a
50	7.2 $\pm$ 0.4 ^a	448 $\pm$ 17 ^{a,b}	2.0 $\pm$ 0.4 ^a
100	7.6 $\pm$ 0.3 ^{a,b}	435 $\pm$ 19 ^{a,b}	1.6 $\pm$ 0.2 ^a
200	8.4 $\pm$ 0.3 ^b	472 $\pm$ 30 ^b	1.7 $\pm$ 0.3 ^a
90 days of administration			
0	6.8 $\pm$ 0.6 ^a	541 $\pm$ 33 ^a	0.9 $\pm$ 0.2 ^a
50	7.0 $\pm$ 0.6 ^a	527 $\pm$ 30 ^b	1.0 $\pm$ 0.2 ^a
100	6.6 $\pm$ 0.4 ^a	555 $\pm$ 20 ^a	1.3 $\pm$ 0.3 ^a
200	7.2 $\pm$ 0.3 ^a	548 $\pm$ 36 ^a	0.8 $\pm$ 0.2 ^a
120 days of administration			
0	6.9 $\pm$ 0.2 ^a	354 $\pm$ 10 ^a	1.4 $\pm$ 0.2 ^a
50	8.1 $\pm$ 0.3 ^b	391 $\pm$ 18 ^b	1.8 $\pm$ 0.4 ^b
100	7.3 $\pm$ 0.6 ^{a,b}	411 $\pm$ 9 ^{b,c}	1.6 $\pm$ 0.4 ^a
200	7.5 $\pm$ 0.1 ^{a,b}	440 $\pm$ 8 ^c	1.0 $\pm$ 0.2 ^a

a-c Values with same superscript are not significantly different
(P>0.05).

Table V-8

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed					Remarks	Reference
					CNS	Blood	Liver	Kidney	Lung		
-DCB	Rat	18.8, 188 or 376 mg/kg/day	5 days/week, 138 doses 192 days	Oral			+	+		No adverse effects at 18.8 mg/kg. Slight increase in kidney and liver weight at 188 mg/kg. Moderate increase in liver weight and cloudy swelling, decrease in spleen weight at 376 mg/kg.	Hollingsworth, et al. (1958)
	Rat	0.001, 0.01 or 0.1 mg/kg day	9 mos.	Oral	+	+	+	+	+	No adverse effects at 0.001 mg/kg. Dose-dependant changes in conditioned reflexes, depression of hematopoietic system at 0.01 and 0.1 mg/kg.	Varshavskaya (1968)
	Rat (20) Guinea pig (8) Mouse (108)	49 ppm	7 hrs/day; 5 days/week; 6-7 months	Inhalation						No adverse effects observed	Hollingsworth, et al. (1958)
	Rat (20) Guinea pig (8) Rabbit (9) Monkey (28)	93 ppm	7 hrs/day; 5 days/week; 6-7 months	Inhalation						No adverse effects observed	"

() = Number of animals tested

Tabel V-8 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed					Remarks	Reference
					CNS	Blood	Liver	Kidney	Lung		
p-DCB	Guinea pig	125 or 250 mg/day as 50% soln or 125 mg/kg in olive oil.	10-11 days	Intra-muscular (I.M.)	+	+				Intense steatosis of liver; weight loss; decreased hepatic glycogen	Frada and Cali, (1958)
	Guinea pig	125 mg/kg	20 days	I.M.	+		+			Weight loss; serum transaminase increased	Totaro, (1961)
	Guinea pig	125 (m)g/ (kg)	20 days	I.M.	+	+				11.4% weight loss; serum transaminase increased	Totaro and Licari (1964)
	Guinea pig	125 (m) g/ (kg)	21 days	I.M.			+			Increase in reaction and clotting formation time	Coppola, et al. (1963)
	Rat	10, 100 or 500 mg/kg as 10% soln.	5 days/week; 20 doses	Oral			+	+	+	No adverse effects observed at 10 or 100 mg/kg; cloudy swelling and centrilobular necrosis of liver; cloudy swelling of renal tubular epithelium	Hollingsworth, et al. (1956)

Table V-8 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	CNS	Blood	Liver	Kidney	Lung	Bone Marrow	Remarks	Reference
	Pekin duck	0.5% in Diet	35 days	Oral							Growth retardation; no cataracts; 3 deaths after 4 weeks on diet	Hollingsworth, et al. (1956)
	Rabbit	500 mg/kg day	5 days/week; 263 doses	Oral			+				Swelling and focal necrosis in liver	"
		1000 mg/kg day	5 days/week; 92 doses over 219 days	Oral	+	+	+				Weight loss; tremors and weakness; necrosis and cirrhosis in liver	"
	Rat	18.8, 188 or 376 mg/kg/day	5 days/week; 138 doses in 192 days	Oral			+		+		No adverse effects observed at 18.8 mg/kg.	"
											Increased liver and kidney weights at 188 and 376 mg/kg.	
											Decreased spleen weight; liver necrosis and cirrhosis at 376 mg/kg.	

Table V-8 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed CNS Blood Liver Kidney Lung Bone Marrow	Remarks	Reference
Rat (9M, 9F)		100 mg/l (16,000 ppm)	20 min/day; 5-9 days	Inhalation	+	Granulocytopenia in 8/18; tendency toward same in 3/18	Zupko and Edwards (1949)
Guinea pig (9M)					+	Granulocytopenia in 5/9; tendency toward same 2/9; weight loss in 6/9	"
Rabbit (18M)			30 min/day		+	Granulocytopenia in 11/18 Irritation of mucosa, weight loss in 14/18; Tremors rapid but labored breathing, death 12/18.	
Rat (10M)		96 ppm,	7 hrs/day; 5 days/week; 16 days	Inhalation	+	No adverse effects observed	Hollingsworth et al. (1956)
(10)		158 ppm	"		+	Cloudy swelling or granular degeneration of liver	"
(5)		173 ppm	"		+	Slight interstitial edema and congestion in lung; slight increase in liver and kidney weights.	"

Table V-8 (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed				Remarks	Reference
					CNS	Blood	Liver	Kidney	Lung	
									Bone Marrow	
	(19M) (15F)	798 ppm	8 hrs/day; 5 days/week; 1-46 doses (M) 9-69 doses (F)	+	+	+	+	+	Tremors; weakness; cloudy swelling of liver (M&F) and kidney (F), Deaths: 2M, 2F	"
	(20M)	341 ppm	7 hrs/day; 5 days/week, 6 months	+	+	+	+	+	Slight increase in liver and kidney weights	"
	Guinea pig (5)	173 ppm	7 hrs/day; 5 days/week; 16 days	+	+	+	+	+	Slight decrease in spleen weight, slight edema and congestion in lungs	Hollingsworth et al. (1956)
	(16M) (7F)	798 ppm	8 hrs/day; 5 days/week 1-23 doses (M) 11-20 doses (F)	+	+	+	+	+	Deaths: 2M	"
	(8)	96 ppm	7 hours/day; 5 days/week 157-219 days						No adverse effects observed	"
	(8)	158 ppm	"					+	Increased liver weight (F); Body weight loss	"

Table V-o (Continued)

Summary of Subacute to Longer-term Toxicity Data

Chemical	Species	Dose	Duration	Route	Effects Observed					Remarks	Reference
					CNS	Blood	Liver	Kidney	Lung		
	(8M) (8F)	314 ppm	7 hours/day; 5 days/week; 6 months		+		+			Cloudy swelling, fatty degeneration, focal necrosis, slight cirrhosis of liver	"
	Rabbit (1M) (1F)	173 ppm	7 hours/day; 5 days/week; 16 days					+		Slight edema and congestion of lungs	"
	(8M) (8F)	798 ppm	8 hours/day; 5 days/week; 1-62 exposures		+		+	+		Reversible non- specific eye changes	Hollingsworth et al. (1956)
	(1M) (1F)	158 ppm	7 hours/day; 5 days/week; 157-219 days							No adverse effects observed	"
	Mouse	96 ppm	7 hours/day; 5 days/week; 157-219 days							No adverse effects observed	"
	(10)	158 ppm	"							No adverse effects observed	"
	Monkey (18)	158 ppm	"							No adverse effects observed	"

Cloudy swelling,
fatty degeneration,
focal necrosis,
slight cirrhosis of
liver

Slight edema and
congestion
of lungs

Reversible non-
specific eye
changes
Hollingsworth
et al. (1956)

No adverse effects
observed

No adverse effects
observed

No adverse effects
observed

No adverse effects
observed

Hollingsworth, et al. (1958) also measured the effects of multiple inhalation exposures of o-DCB on rats, guinea pigs, mice, rabbits and monkeys. A range of concentrations was used, seven hours a day, five days a week, for six to seven months. No adverse effects were observed in rats, guinea pigs or mice exposed to 49 ppm, or in rats, guinea pigs, rabbits and monkeys exposed to 93 ppm (0.56 mg/l).

Oral administration of o-DCB to white rats for nine months was conducted at doses of 0.001, 0.01 or 0.1 mg/kg/day (Varshavskaya, 1968). Effects were observed at the two higher doses similar to those described for monochlorobenzene. The author reported an inhibition of mitosis in the bone marrow, as well as neutropenia and abnormal conditioned reflexes. These changes in the blood profile may be important in that they could be precursors to pancytopenia or leukemia. In this report, however, Varshavskaya concluded that no carcinogenic activity was observable at the doses studied. She measured tissue DPN, TPN, glucose-6-phosphate and alkaline phosphatase since it has been claimed that an increase in the activity of these enzymes is indicative of carcinogenicity (Burstone, 1965). At the 0.1 and 0.01 mg/kg doses, o-DCB caused an increase in acid phosphatase but a decrease in alkaline phosphatase.

The 0.1 mg/kg dose of o-DCB caused a marked increase in the amount of 17-ketosteroids found in the urine. This increase is most likely due to hyperplasia of the adrenal cortex, since an increase in the weight of the adrenals and decrease in the ascorbic acid concentration of the adrenals also were observed. The 0.001 mg/kg dose had no observable effects on any of the parameters studied.

Para-Dichlorobenzene

Intramuscular injection of 125 or 250 mg p-DCB/kg into guinea pigs over a 10 or 20 day period resulted in effects typical of p-DCB toxicity (Frada and Cali, 1958; Totaro, 1961; Totaro and Licari, 1964; Coppola, et al., 1963). The observed changes included weight loss, liver changes and blood clotting time increases.

Oral doses of 10, 100 or 500 mg p-DCB/kg, five days a week, for 20 doses produced marked cloudy swelling and necrosis in the central area of the liver lobules only with the highest dose (Hollingsworth, et al., 1956). No effects were observed at the other doses. Pekin ducks, fed 0.5% p-DCB in their diets for 35 days exhibited depression of body weight gain. Three animals died after the fourth week.

Oral doses of 188 or 376 mg p-DCB/kg, five days a week, for 192 days (138 doses) in rats induced an increase in the weights of the liver and kidneys (Hollingsworth, et al., 1956). At 376 mg/kg, increased splenic weight, slight cirrhosis and focal necrosis of the liver also were observed. There was also cloudy swelling of the renal tubular epithelium. No adverse effects were seen with the 18.8 mg/kg dose. Rabbits receiving 500 mg/kg/day oral doses, 5 days a week, for a total of 263 doses showed swelling and focal necrosis of the liver. Other rabbits receiving a total of 92 oral doses at 1000 mg/kg/day over a 219-day period exhibited necrosis and cirrhosis of the liver. CNS effects were evident as well, as both tremors and weakness were noted. Loss of body weight also occurred.

The toxicity of p-DCB also has been studied via the inhalation route of exposure. Zupko and Edwards (1949) exposed

rats, guinea pigs and rabbits to a high level of compound (100 mg/l or 16,000 ppm) 20-30 minutes/day for 5-9 days. In all species, adverse effects were observed on the CNS, liver, kidney and lung. Among the rats (9 males and 9 females), eight animals exhibited granulocytopenia, with three others showing a tendency toward the same. In the guinea pigs (9 males), granulocytopenia was observed in five, with a tendency towards this condition in two others. In addition, six of the nine showed a weight loss. Eleven of the 18 male rabbits exposed developed granulocytopenia. Weight loss and mucosal irritation occurred in 14 of the 18. CNS effects manifested as tremors and rapid and labored breathing also occurred. Twelve of the 18 animals died.

Hollingsworth, et al. (1956) conducted a series of inhalation studies with p-DCB, at various levels and durations of exposure, in rats, guinea pigs, monkeys, rabbits and mice. The concentrations used were 96, 158, 173, 314 and 798 ppm (0.58, 0.95, 1.04, 2.05 and 4.8 mg/l, respectively). Rats exposed to 96 ppm seven hours a day, five days a week, for 16 days showed no abnormalities. A 157-219 day exposure at this level in guinea pigs and mice yielded no adverse reactions. With the same protocol, inhalation levels of 158 and 173 ppm caused cloudy swelling or granular degeneration of the liver, slight increase in the weight of the liver and kidneys and some interstitial edema and congestion in the lungs of rats and guinea pigs. Monkeys and mice showed no adverse effects at these levels. Rats exposed to 341 ppm for six months showed evidence of a slight increase in the weight of the liver and kidneys. Guinea pigs exposed to 173 ppm for 16 days showed a slight decrease in

splenic weight and some lung edema and congestion. At 314 ppm, for six months, the liver became slightly cirrhotic with focal necrosis, cloudy swelling and fatty degeneration. Rabbits, exposed to an atmosphere of 158 ppm for 157-219 days, were unaffected. At 173 ppm for 16 days, slight edema and lung congestion were observed, and at 798 ppm, for 1-62 exposures, reversible non-specific eye changes were apparent.

Effects Upon Drug-Metabolizing Enzymes

Most mammals possess a group of enzymes that specializes in the biotransformation of foreign compounds. These enzymes are located in the endoplasmic reticulum of the liver cells. The metabolic transformation of foreign compounds usually leads to the conversion of lipophilic materials into more polar compounds, which are eliminated more readily from the hepatocyte and excreted from the body. Thus, compounds which are of little nutritive value are prevented from accumulating in cells and tissues. In this way, undesired effects may be avoided (see reviews in Williams, 1959; Parke, 1968).

The endoplasmic reticulum (ER) of the cell is the location of many enzymes such as glucose-6-phosphatase, glucuronyl transferase, the hydroxylases and protein synthetases (Parke, 1972). The enzymes concerned with the metabolism of drugs and other xenobiotics are referred to frequently as mixed function oxidases or monooxygenases. All of the monooxygenase drug metabolizing enzymes require reduced nicotinamide adenine dinucleotide phosphate (NADPH₂), molecular oxygen and a cytochrome, usually P-450. The ER is associated not only with the oxidation of drugs, but also the

biosynthesis of cholesterol, the catabolism of bile acids, the oxidation of fatty acids and the oxidation of prostaglandins. In addition to mixed function oxygenase activities, the hepatic endoplasmic reticulum contains a number of reductases, some of which utilize cytochrome P-450 and NADPH₂ (Williams, 1959; Parke, 1968).

The biotransformation of drugs and xenobiotics appears to occur in two distinct phases. The first phase includes reactions classified as oxidations, reductions and hydrolyses. In the second phase, the reactions are referred to as syntheses or conjugations. These enzyme reactions, especially oxidations which require cytochrome P-450, have been examined throughout the animal kingdom. Contrary to the suggestion of an evolutionary trend for the appearance of cytochrome P-450 in mammals, this cytochrome is apparently ubiquitous (Ahokas, et al., 1976).

However, quantitative differences in metabolism exist, for example, among livers from various species, among various tissues from a single animal, and between the neonate and adult within a species. It also has been well documented that enzymes of biotransformation may be regulated (stimulated or depressed) by xenobiotics or steroids (Parke, 1972). While there does appear to be a ubiquitous distribution of cytochrome P-450, the concentration in different animal species varies greatly, as can be seen in Table V-9. Since these species have differing amounts of cytochrome P-450, they must have different abilities to manufacture toxic intermediates. They also have varying abilities to metabolize benzpyrene and halogenated benzenes.

A number of investigators have suggested that a relationship exists between the induction of δ -aminolevulinic acid synthetase and the induction of drug or xenobiotic metabolizing systems by compounds which are known to induce liver porphyria (Rimington and Ziegler, 1963; Poland, et al., 1971; Ariyoshi, et al., 1975). The effects of a series of chlorinated benzenes on hepatic δ -ALA synthetase and cytochrome P-450 levels are summarized in Table V-10.

Poland et al. (1971) treated young female Sherman rats by gavage with daily doses of m-DCB in peanut oil. Continuous daily dosing with 800 mg/kg/day for nine days resulted in a biphasic pattern of urinary coproporphyrin excretion (Figure V-6). ALA synthetase was measured in animals dosed daily with 800 mg/kg/day for 1, 3 or 5 days. Enzyme activity measured 24 hours after the last dose showed a steep rise after Day 1, with lesser increases seen after Days 3 and 5 (Table V-11). Increases in liver size were not sufficient to account for the changes observed. No histological evidence of liver damage was noted in these animals.

The cyclic nature of the response suggests that an adaption or tolerance to the compound is developing, perhaps because the animal may be detoxifying the substance more rapidly. This possibility was tested by observing the effects of the same dosing regimen as described above on hexobarbital (150 mg/kg i.p) sleeping time. Sleeping times were significantly shortened after one dose of m-DCB (from 180 minutes to less than 120 minutes), and after three days, were only 20% of control times (180 minutes) vs. 30 minutes ($P < 0.001$). Acceleration of the rate of metabolism of bishydroxycoumarin (BHC) also occurred after treatment with m-DCB. After

Table V-10

Effects of Chlorinated Benzenes on Aminolevulinic Acid
Synthetase and Cytochrome P-450 Content of Rat Liver

(Rimington and Ziegler, 1963; Poland et al., 1971; Ariyoshi et al., 1975).
(Modified from Ware and West, 1977)

Compound	Extent of metabolism	P-450 n moles/g	ALA Synthetase n moles/g/hr	Prophyria [†]
Control *		0.68	22.6	
Monochlorobenzene	High	0.56	49.2	yes (1140)
1,2-Dichlorobenzene	High	0.66	36.3 ⁺	yes (455)
1,3-Dichlorobenzene	High	0.77	29.8 ⁺	
1,4-Dichlorobenzene	High	0.73	32.3	yes (770)
1,2,3-Trichlorobenzene	High	0.84	33.6	yes (785)
1,2,4-Trichlorobenzene	High	1.63	47.5 ⁺	yes (730)
1,3,5-Trichlorobenzene	Low	0.73	34.8	-
1,2,3,4-Tetrachlorobenzene	High	0.97	33.3 ⁺	yes (660)
1,2,3,5-Tetrachlorobenzene	Low	1.14	35.4 ⁺	-
1,2,4,5-Tetrachlorobenzene	Low	0.99	27.5	-
Pentachlorobenzene	Very low	1.16	98.7 ⁺	-
Hexachlorobenzene	Very low	0.97	51.8	yes (400)

* Rats were pretreated orally with 250 mg/kg body wt. for three days and were sacrificed 24 hours after the last dose.

+ Significantly increased over control.

† Numbers in parentheses indicate dose in mg/kg body wt. used to produce porphyria in 5 days. (Note: glutathione reduces porphyria produced by halogenated benzenes.)

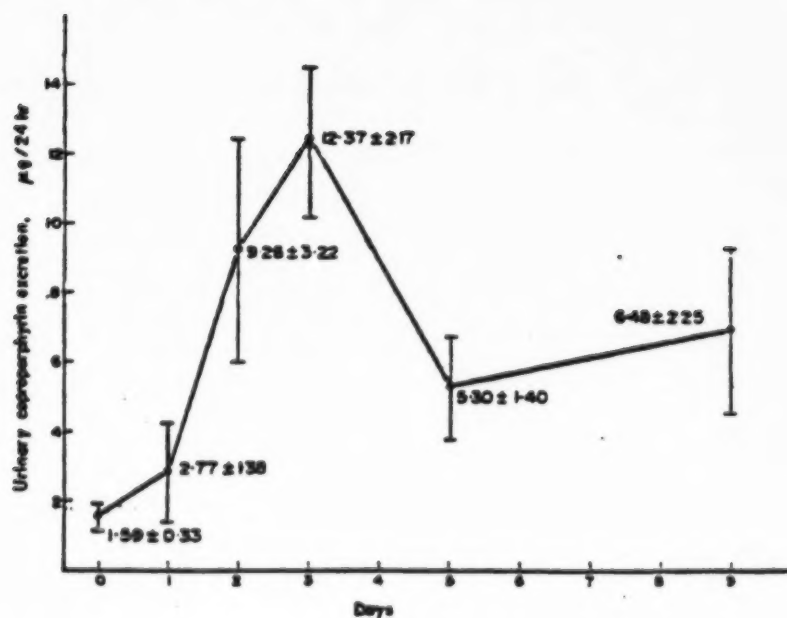


Fig. V-6. Effect of m-DCB on urinary coproporphyrin excretion. Four female rats (90-120 g) were treated daily with 800 mg/kg m-DCB. The 24-hr urinary excretion of coproporphyrin (mean $\pm$ S.D.) is plotted versus time. The first dose was given on day 0, and the urinary coproporphyrin value for each day represents a urinary collection for the previous 24 hr. Urinary coproporphyrin excretion was significantly lower on days 5 and 9 than on day 3.

Source: Poland, et al. (1971).

Table V-11

Effect of m-Dichlorobenzene on ALA
Synthetase Activity*

Treatment	ALA synthetase (nmol/g/hr)		
	Day 1	Day 3	Day 5
Control	13.3 $\pm$ 4.4 (7)	18.8 $\pm$ 4.6 (6)	16.1 $\pm$ 4.8 (7)
m-DCB	52.9 $\pm$ 15.3 (7)	40.5 $\pm$ 6.8 (7)	30.5 $\pm$ 6.2 (8)

* Female rats were treated orally for 1, 3 or 5 days with m-DCB (800 mg/kg) or peanut oil and sacrificed 24 hr after the last dose. All values represent the means $\pm$ S.D. The number of animals in each group is given in parentheses.

ALA synthetase activity is lower on day 5 in m-DCB-treated animals than on day 1 ($P < 0.01$) or day 3 ($P < 0.02$).

Source: Poland, et al. (1971)

five days of dosing, the serum BHC levels measured 82 ± 24 ug/ml, as compared with control levels of 133 ± 10 ug/ml ($P < 0.025$).

Poland et al. (1971) conducted further experiments designed to test the hypothesis that m-DCB stimulates its own metabolism. Liver and serum levels of m-DCB and 2,4-dichlorophenol (DCP), its major metabolite, were determined after dosing daily for up to five days, as described above. The serum m-DCB concentration was higher on Day 3 (8.89 ± 1.61 ug/ml) than on Day 1 (3.25 ± 1.31 ug/ml), but was significantly lower on Day 5 (5.91 ± 2.02 ug/ml) than on Day 3 ($P < 0.02$). The hepatic concentration rose steeply from Day 1 (13.01 ± 6.92 ug/g tissue) to Day 3 (44.17 ± 6.31 ug/g). However, the difference between the concentration at Day 3 and 5 (32.1 ± 16.18 ug/g) was not quite statistically significant ($0.10 > P > 0.05$). In rats pretreated for four days with 40 mg/kg phenobarbital i.p., a known inducer of drug metabolism, before receiving a single 800 mg/kg dose of m-DCB, slightly lower concentrations of m-DCB in liver (8.49 ± 2.37 ug/g) and serum (2.24 ± 0.49 ug/g) were observed as compared with those in rats receiving only a single dose of m-DCB. The differences were not significantly different, however. The data from this series of experiments add support to the hypothesis that m-DCB does, in fact, stimulate its own metabolism.

Ariyoshi et al. (1975) studied changes in certain liver constituents, cytochrome contents, activities of some drug-metabolizing enzymes and Δ -ALA synthetase in rats treated with each of the three isomers of DCB. Animals received oral doses of 250 mg/kg/day for up to three days. Activities of aminopyrine demethylase and aniline hydroxylase were enhanced markedly by m-DCB, but cytochrome

content was not altered significantly after any of the three isomers. Delta-ALA synthetase activity was increased significantly by treatment with o-, m- and p-DCB (63%, 32% and 42%, respectively). However, significant parallel changes in the cytochrome P-450 content did not occur. All isomers increased microsomal protein content of liver preparations. Microsomal inorganic P content was also increased by 36% after treatment with m-DCB.

Carlson and Tardiff (1976) studied the effects of chlorobenzene, 1,4-dichlorobenzene, 1-bromo-4-chlorobenzene, 1,2,4-trichlorobenzene and hexachlorobenzene on adult male rats for 14 days at low doses from 10 to 40 mg/kg body weight. All halogenated benzenes except monochlorobenzene decreased hexobarbital sleeping time immediately and/or 14 days following treatment. As can be seen in Table V-12, cytochrome-c reductase, cytochrome P-450 content, O-ethyl O-p-nitrophenyl phenylphosphonothioate (EPN) detoxication, glucuronyl transferase, benzpyrene hydroxylase and azoreductase were increased to varying degrees. Administration of 1,4-di- and 1,2,4-trichlorobenzene for 90 days resulted in an increase in EPN detoxication, benzpyrene hydroxylation and azoreductase. The increases were still apparent 30 days later. 1,2,4-Trichlorobenzene was the most potent inducer of cytochrome reductase and cytochrome P-450. Either no change or a decrease was reported for glucose-6-phosphatase and isocitrate dehydrogenase activities. .

These findings demonstrate the ability of halogenated aromatic compounds at low doses to induce enzyme systems associated with the metabolism of foreign compounds. This type of action may influence the metabolism of endogenous steroids, other foreign compounds and drugs.

Table V-12⁹

Effect of Chlorinated Benzenes Administered Orally for 14 Days on Various Parameters of Xenobiotic Metabolism

(Carlson and Tardiff, 1976)
(Modified from Ware and West, 1977)

Compound	Dose (mg/kg/day)	Cytochrome c reductase (nmol cytochrome c reduced/min mg protein)*	Cytochrome p-450 (E/mg protein x 10)	Glucuronyl- transferase (nmol/min/mg protein)	EPN [†] detoxication (pg p-nitrophenol/ 50 mg/30 min)	Benzpyr- hydroxy (nmol/m pro)	Azoreduc- tase (ng/min/mg protein)
Monochloro- benzene	0	100 + 25	236 + 20	5.8 + 0.4	6.7 + 0.6	1.39 + 0.43	
	200	110 + 8	209 + 10	9.4 + 0.6	7.1 + 0.5	0.89 + 0.05	
	400	109 + 9	197 + 14	12.2 + 0.3	8.4 + 0.9	0.92 + 0.04	
	800	78 + 5	133 + 15	13.0 + 0.5	7.4 + 0.7	0.78 + 0.16	
1,4-Dichloro- benzene	0	156 + 9	178 + 20	5.9 + 0.6	6.7 + 0.6	2.36 + 0.49	63.9 + 2.8
	10	151 + 6	174 + 15	7.8 + 0.6	7.2 + 0.6	1.75 + 0.13	67.5 + 2.5
	20	165 + 8	167 + 11	11.3 + 1.0	9.5 + 1.1	3.53 + 0.68	71.4 + 4.3
	40	176 + 10	193 + 13	9.6 + 0.6	9.0 + 0.5	2.15 + 0.34	79.2 + 6.6
1-Bromo-4- chlorobenzene	0	149 + 22	205 + 35	5.8 + 1.4	9.5 + 0.8	1.51 + 0.20	59.1 + 2.5
	10	126 + 6	180 + 7	5.6 + 0.8	11.4 + 0.9	1.37 + 0.17	60.9 + 2.6
	20	123 + 4	202 + 7	9.0 + 0.7	12.4 + 1.1	2.06 + 0.26	69.7 + 4.2
	40	141 + 5	245 + 19	9.1 + 0.5	14.4 + 0.6	2.35 + 0.23	79.4 + 2.7
1,2,4-Tril- chlorobenzene	0	103 + 8	72 + 13	5.0 + 0.4	5.8 + 0.3	2.82 + 0.53	72.4 + 11.1
	10	139 + 15	99 + 18	5.1 + 0.4	10.3 + 0.9	3.82 + 0.65	102.0 + 2.7
	20	192 + 12	212 + 45	8.6 + 1.6	12.0 + 0.8	5.37 + 1.01	91.1 + 4.6
	40	183 + 9	268 + 15	8.3 + 0.7	15.4 + 0.4	5.22 + 1.14	130.7 + 5.3
Hexachloro- benzene	0	85 + 7	128 + 18	6.3 + 1.0	5.3 + 0.9	2.79 + 0.54	122.0 + 24.9
	10	106 + 10	204 + 18	4.5 + 0.5	15.0 + 1.1	4.76 + 0.58	287.7 + 7.4
	20	109 + 7	190 + 16	6.8 + 0.3	18.4 + 0.8	4.08 + 0.37	210.0 + 7.4
	40	100 + 7	150 + 27	6.6 + 0.8	18.9 + 0.7	4.24 + 0.82	262.7 + 19.1

* Value is mean + S.E. for group of six rats except for benzpyrene hydroxylase group receiving 40 mg/kg of 1,2,4-trichlorobenzene. In that group, there were five rats.

Multiple Chemical Exposures: Actions of Combinations

Since many compounds, whether they are agricultural and industrial chemicals or drugs, are handled similarly by the cytochrome P-450 system, there would be many possibilities for additive, synergistic as well as antagonistic actions.

Experiments to determine this should be carefully designed. For example, in man, phenobarbital has been shown to stimulate drug metabolism. This effect requires several days to reach a maximum rate (Goodman and Gilman, 1975). If treatment continues, it appears that the marked stimulation is lost. This has not been taken into consideration in many animal studies, i.e., only the three to four day effect has been studied.

From recent studies in man using identical or fraternal twins, the role of the environment as an explanation for differences in metabolism is being deemphasized in favor of genetic differences. It has been postulated that people who have genetic susceptibility to inducible drug-metabolizing enzymes may be more prone to adverse effects, if there is a toxic intermediate or product formed in vivo (Goujon et al., 1972; Vesell et al., 1976). Goujon et al. (1972) found that hexachlorobenzene differentially inhibits aryl hydrocarbon hydroxylase in genetically responsive and non-responsive mice. Similarly, Vesell et al. (1976) showed that the toxicity of chloroform to the kidney is different in genetically susceptible and non-susceptible mice.

Goujon et al. (1972) and Vesell et al. (1976) demonstrated genetically controlled variations in the susceptibility of mice to hexachlorobenzene and chloroform. Hence, exposure to a wide range of

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Goujon et al. (1972) and Vesell et al. (1976) demonstrated genetically controlled variations in the susceptibility of mice to hexachlorobenzene and chloroform. Hence, exposure to a wide range of

environmental contaminants including the halogenated benzenes could affect the ability of these animals to detoxify xenobiotic substances. Synergistic effects of the halogenated benzenes in combination or with other environmental contaminants could be especially damaging for the genetically susceptible individual.

There was one example of synergistic effects of halogenated benzenes on a target organism found in the literature (Hinze, et al., 1970). The antifungal activity of halogenated benzenes was synergistic with organo-tin compounds. The mechanism of the toxicity was not discussed. There were no studies found on synergistic effects in mammals or other nonmammalian species.

Teratogenicity

No teratogenicity studies with the dichlorobenzenes alone were found in the peer-reviewed literature. Studies to assess the embryotoxic and teratogenic potential of o-DCB and p-DCB have been initiated and/or completed under sponsorship of the chemical industry.

Hodge, et al. (1977) conducted a teratogenicity study of p-DCB in rats. Groups of pregnant rats (32 animals per group) were exposed to atmospheric concentrations of 0, 75, 200 or 500 ppm p-DCB 6 hours/day on Days 6-15 of pregnancy. Data were collected only from the first 20-24 animals in each group proven to be pregnant at the time of sacrifice (Day 21). Nine animals littered spontaneously on Day 21 and thus were not included in the study results (two at 75 ppm, two at 200 ppm and five at 500 ppm).

Maternal weight gain was monitored over the 21 days of pregnancy. Exposure to p-DCB did not alter the rate of weight gain in any group when compared with controls.

On Day 21, the animals were sacrificed. The intact uteri were examined for numbers of fetuses and resorptions. Corpora lutea were counted. Upon dissection of the uteri and removal of the fetuses, the resorptions which had occurred were classified as early or late. Resorptions are designated as late when fetal tissues are distinguishable. When abnormal fetuses were noted, maternal heart, liver, lung, uterus, ovary, kidney, and adrenal were preserved for histological examination. Liver and lung from at least ten animals/group also were fixed for histology.

Each fetus was examined for viability, sex, weight and presence of abnormalities. Half of each litter was eviscerated, examined for abdominal abnormalities and stained with Alizarin Red for subsequent skeletal examination for abnormalities and degree of ossification.

Upon gross examination, uteri from three females exposed to 75 ppm contained excessive amounts of blood. In one case, this appeared to be associated with a dead fetus. One female exposed to 200 ppm had inflated lungs. Upon histological examination, no lesions were observed which were attributable to exposure to p-DCB.

Exposure to p-DCB did not induce adverse effects on numbers of implantations, viable fetuses, resorptions, corpora lutea or on mean fetal weights, mean litter weights or on implantation efficiency (number of implantations/number of corpora lutea). Sex ratios (male/female) were within normal limits. There was no increase in the

number of runts.

Three gross fetal abnormalities were noted, one in each experimental group. From the 75 ppm group, there was one fetus with gastroschisis and malrotation of the left hindlimb. In the 200 ppm group, there was a single fetus with gastroschisis and malrotation of the right hindlimb, and in the 200 ppm group, there was one fetus with agnathia and cleft palate. One control fetus was found to be anemic.

Upon examination for skeletal abnormalities, no evidence was found that maternal exposure to p-DCB resulted in delayed ossification of fetal bones or an increased incidence of minor abnormalities. Occasionally, 14 ribs were noted, but, in almost all cases, these were vestigial.

The results of this study suggest that maternal exposure to atmospheric levels of p-DCB up to 500 ppm on Days 6-15 of pregnancy does not result in any embryotoxic, fetotoxic or teratogenic effects in the offspring.

Dow Chemical is completing studies in which pregnant rats and rabbits are being exposed to o- and p-DCB via inhalation. Final reports are not yet available to ODW. However, when they are, they will be subjected to review and evaluation. Dow Chemical, however, has submitted a review of the results of the dose range-finding study to determine the maximum tolerated dose of o-DCB for the full study, as well as the final protocol for that study (Dow, 1981). In the probe study, groups of 10 pregnant rats and seven pregnant rabbits were exposed to nominal o-DCB concentrations of 0, 200, 400, or 500 ppm (0, ~1200, ~2400 or ~3000 mg/m³). The

animals were exposed six hours/day on Days 6-15 (rats) or Days 6-18 (rabbits) of gestation.

Severe maternal toxicity, as evidenced by significant decreases in body weight, body weight gain and food consumption, increases in relative liver and kidney weights and signs of systemic toxicity at gross necropsy, was observed in pregnant rats exposed to 500 ppm of o-DCB. Embryoletality, secondary to maternal toxicity, was observed among rats in the 500 ppm exposure group. Increased relative liver and kidney weights and decreased food consumption were observed among pregnant rats exposed to 400 ppm of o-DCB. Exposure to 200 ppm of o-DCB did not produce any sign of toxicity among maternal animals. No statistically significant effects on reproductive parameters were observed among rats at any exposure level.

Among rabbits, slight maternal toxicity was observed among pregnant animals exposed to 500 ppm of o-DCB. Non-significant decreases in maternal body weight gain, and absolute and relative liver weights were observed among rabbits exposed to 500 ppm. Gross observation at necropsy revealed hepatic changes indicative of mild toxicity among pregnant rabbits exposed to 500 ppm. No adverse effects were observed among rabbits exposed to 200 or 400 ppm of o-DCB, and no significant effects on reproductive parameters were observed among rabbits at any exposure level.

Based on the results of the probe study, as summarized above, exposure concentrations of 0, 100, 200 or 400 ppm o-DCB were chosen as the test concentrations for the definitive teratology study in rats and rabbits.

Recently, Dow Chemical submitted to EPA the results of the dose range-finding study for p-DCB exposure to pregnant rabbits (Hayes, et al., 1982). Pregnant females were exposed to 0, 300, 600 or 1,000 ppm (0, 1,800, 3,600 or 6,000 mg/m³). Each was exposed for 6 hrs/day on Days 6-18 of gestation.

No maternal deaths occurred during the study and no significant changes in gross appearance or demeanor were observed among p-DCB-exposed rabbits. Evidence of slight maternal toxicity was observed among pregnant rabbits exposed to 1,000 ppm of DCB. In this group, a decrease in body weight gain and slight decreases in absolute and relative liver weights were observed. In addition, histopathologic examination of livers revealed decreased hepatocellular vacuolization suggestive of decreased glycogen deposition in the 1,000 ppm group.

No significant effects on the incidence of implantations undergoing resorption were observed in any of the exposed groups when compared to controls indicating that p-DCB is not embryolethal at exposure concentrations up to 1,000 ppm.

Based on the results of this probe study, where evidence of slight maternal toxicity was observed in the 1,000 ppm group, exposure levels of 100, 300 and 800 ppm of p-DCB were selected for the definitive teratology study in rabbits.

Mutagenicity

Effects on Plants

Abnormal mitotic division of the onion, Allium cepa, after treatment with several halogenated benzenes has been described by Ostergran and Levan (1943). Ortho-DCB produced full c-mitosis abnormalities at 300×10^{-6} mol concentration with partial disturbances of mitotic division at 100×10^{-6} mol.

Para-dichlorobenzene also induces abnormal mitotic division in higher plants (Ostergran and Levan, 1943). Effects seen include shortening and thickening of chromosomes, precocious separation of chromatids, tetraploid cells, binucleate cells and chromosome bridges (c-mitosis). Available studies with p-DCB are summarized in Table V-13.

Effects on Microorganisms

Anderson et al. (1972) evaluated 110 herbicides for their ability to produce point mutations in a number of different microbial systems. When tested in a culture of histidine-requiring mutants of Salmonella typhimurium, both o-DCB and trichlorobenzene (isomer not specified) gave a negative response, i.e., they were not mutagenic. Pentachlorophenol, a metabolite of pentachlorobenzene, also was negative in this test system. The metabolites of other halogenated benzenes were not evaluated. No liver homogenates containing the metabolic activating enzymes were added in order to study the effect of conversion of the test compounds to active intermediates.

Prasad and Pramer (1968) and Prasad (1970) investigated the mutagenic effects of the three dichlorobenzene isomers. The chemicals were evaluated for frequency of back mutation of the

Table V-13

Effects of p-DCB on Mitotic Division of Plants
(Modified after Ware and West, 1977)

Organism	Treatment	Fragments	Persistence Of Fragments	Remarks	Reference
<u>Allium</u>	0.05, 0.1, 0.25, 0.5, 1.5 g for five days to seeds in Petri dish same doses to four-day old seedlings for four hours			No signs of mitosis at three highest doses Polyploidy in root cells at 0.5 and 1.5 g Abnormal chromo- some numbers; lagging chromo- somes and dumb bell shaped nuclei also occasionally seen	Carey and McDonough, 1943
Six species of monocoty- ledon angio- sperms-root tips	4 1/2 hour soak in sat. soln.	+	+	Frequency of frag- ments at metaphase tended to decrease. Frequency high-24, 48 hrs. and de- creased at 72, 96 hrs. 2 plants still highly fragmented at 96 hrs. 1 complete recovery.	Sharma and Rattacharya, 1956
Nine species of dicotyle- don angio- sperms-root	1-4 hours depending on plant	+	+	2 plants recovered before 96 hrs. 1 plant died after 48 hrs. 3 plants very	Sharma and Phattacharya, 1956

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Table V-13 (Continued)

Organism	Treatment	Fragments	Persistence Of Fragments	Remarks	Reference
<u>Nothoscoordum</u> <u>fragrans</u> Kunth					
Root tips	3 1/2 hrs. soak in sat. soln.	+	not indicated	Erosion and frag- mentation of chromo- somes both at meta- phase and anaphase	Sharma and Sarkar, 1957
Root tips	6 hrs. sat.	++	not indicated	Erosion and stick- iness increase in fragments from 3 1/2 hour soak.	Sharma and Sarkar, 1957
Pollen	6 hours	-	-	No fragmentation, some diplochromo- somes. Indication of slight disturbance in spindle mechanism and failure in sepa- ration.	Sharma and Sarkar, 1957
(Flower Buds)	(3 1/2 hrs. sat. soln.)	(-)	(-)	(Meiotic division - only stickiness of chromosomes noted.)	Sharma and Sarkar, 1957
	(6 hrs. sat. sol.)	(-)	(-)	(Lagging, non-dis- junction and stick- iness of chromosomes. No fragments.)	Sharma and Sarkar, 1957
Three species of <u>Viciaeae</u> - root tips	4-6 hours in soln.	+	not indicated	c-Mitosis abnormali- ties. Breaks associ- ated with heterochro- matic chromosome reg- ions	Srivastava, 1966
Pennugreek seeds	4-24 hours soak in sat. soln.	-	-	No change in morph- ology or cytology of seedlings	Gupta, 1972

Table V-13 (Continued)

Organism	Treatment	Fragments	Persistence Of Fragments	Remarks	Reference
Fenugreek seedlings	4 hours "exposure"	-	not indicated	Root tip chromo- somes contracted and arrested at metaphase	Gupta, 1972
	Greater than 4 hours (not speci- fied)	-	-	After "longer" treatment, mitosis appeared normal	Gupta, 1972
Zea Mays L. (corn)	3 hour soak of 0.5 cm long seed- lings in saturated aqueous solution, then growth allowed for 7 more days			Accelerated root growth, cell divi- sion; polyploidy, formation of chromo- somal bridges and laggards at anaphase. Polarity of cell changed to an angle of 90° from normal.	Sharma and Agarwal, 1980
<u>Lens escul-</u> <u>enta micro-</u> <u>sperma</u>	4-48 hour soak of root tips in saturated aqueous solution			Germiability and growth inversely proportional to level and duration of exposure. Mitotic and chromosomal anomalies observed: chromosome contrac- tion and condensa- tion, fragmentation, bridges, tetraploidy, binucleate cells	Sarbhoy, 1980.
	4-48 hour exposure of germinating seeds to vapors of 25, 50, 100, 250, 500, 800-1,000 mg of p-DCB crystals				
	4-48 hour soak of germinating root tips in saturated aqueous solution				

methionine-requiring (meth₃) locus in the fungus Aspergillus nidulans. The mutagenicity of the dichlorobenzenes increased in the following order: o-DCB-5/10⁶ spores, m-DCB-9/10⁶ spores and p-DCB-11/10⁶ spores.

Anderson (1976) reported on a study with p-DCB designed to estimate the mutagenic potential of this substance in the Salmonella typhimurium plate incorporation assay. Mutant strains TA 1535, TA 1538, TA 98 and TA 100 were exposed to varying concentrations of p-DCB dissolved in DMSO in two separate studies. In the first study, concentrations of 100, 500 and 2500 ug/plate were used. The experiment was run three times. In the second study, concentrations of 4 and 20 ug/plate were used in addition to the three used in the first study. This experiment was run five times. Exposure to p-DCB occurred both with and without metabolic activation with S-9 mix from Aroclor-treated rats. In another series of experiments, the tester strains were exposed to atmospheric concentrations of p-DCB at levels of 94, 299 or 682 ppm, again with and without metabolic activation. This protocol was employed four separate times.

A greater than two-fold increase in the number of revertants is the criterion by which a compound is considered to be mutagenic in this assay system. After atmospheric exposure to p-DCB, no significant increases were noted in any tester strains, except in one of the four exposures to 682 ppm in TA 1535 with metabolic activation. This increase was not observed in three follow-up experiments. In the first series of experiments in which p-DCB was dissolved in DMSO at three concentrations, the number of revertants in TA 1535 increased

nearly 10-fold at 500 ug/plate, with metabolic activation. This increase occurred in two of the three runs. This increase was not observed in the subsequent series of five runs employing five doses including the previously-described three. In spite of the few positive results, the data from all of the experiments together would suggest that p-DCB is not mutagenic to tester strains used in this Salmonella typhimurium plate incorporation assay system, either when dissolved in DMSO or in the gaseous phase.

More recently, Simmon et al. (1979) examined all three dichlorobenzene isomers for mutagenic activity in both the standard Ames/Salmonella assay and the E. coli WP2 system. In the Ames assay, tester strains TA 1535, 1537, 1538, 98 and 100 were employed, with and without metabolic activation. In the first of two experiments, concentrations of 0.05-1.0 ul/plate (0.065-1.3 mg/plate) of o- or m-DCB were added to each Salmonella strain or E. coli culture. No reproducible dose-related increases in the number of revertants were observed in either system. In a second experiment, each compound was retested at lower levels, ranging from 0.0005 to 0.5 ul/plate. Again, no significant changes were noted. Para-DCB was tested in the same manner, but at higher dose levels: 50-1,000 ul/plate in the first experiment and 0.5-500 ul/plate in the second. Again, no increases in the number of revertants were observed.

Simmon, et al. (1979) also conducted tests for chromosomal aberrations in yeast. All three isomers were tested for their potential to induce mitotic gene conversion and reciprocal recombination in Saccharomyces cerevisiae C3, with and without metabolic activation. At doses ranging from 0.001-0.25%, o-DCB produced no effects.

Toxicity was observed at 0.05%, with and without activation. Meta-DCB was tested at doses ranging from 0.005 to 0.1% in two experiments. In both, but only after activation, enhancement of the recombination response was observed. Para-DCB, at dose levels ranging from 0.005-5% in the first three experiments, appeared to increase mitotic recombination, with a considerable variation in survival. In two later experiments, toxicity occurred, but no increase in recombination was observed. The inconsistency of the results may be attributed to the relative insolubility of the compound.

The differential toxicity of each of the three isomers was evaluated in the DNA repair-proficient and repair-deficient strains of E. coli (W3110 $\text{polA}^+/\text{p3478 polA}^-$) and Bacillus subtilis (H17 $\text{rec}^+/\text{M45 rec}^-$) (Simmon, et al., 1979). In three of four experiments, 20 ug/plate o-DCB was more toxic to the repair-deficient E. coli (polA^-) than to the repair-proficient strain (polA^+). There was no apparent difference in toxicity to either strain of B. subtilis. At the same concentration (20 ul/plate), m-DCB was more toxic to the repair-deficient strain of E. coli than to the polA^+ strain in four of five experiments. Para-DCB, at concentrations of 1 or 5 mg/plate, had no effect on any of the four strains. This result was interpreted to mean that either the compound was truly nontoxic under these test conditions or that the substance was unable to diffuse away from the impregnated filter paper disc in the culture dish.

In summary, the results of these studies indicate that the dichlorobenzenes do possess mutagenicity activity in certain of the test systems. None were positive in the Ames/Salmonella assay system or in the E. coli WP2 assay. However, m-DCB, both with and without

metabolic activation, increased mitotic recombination in S. cerevisiae. The results with p-DCB were ambiguous. Both o- and m-DCB were shown to interact with and damage bacterial DNA in the E. coli W3110 polA⁺/p3478 polA⁻ differential toxicity assay system.

Effects on Animals

There has been at least one in vitro study of the inhibitory effect of dichlorobenzene (isomer not specified) on the number of mitoses in rat lung cell cultures (Guerin, et al., 1971). The dose of 5 ug did not produce any significantly different number of mitoses than the control. In this test system, dichlorobenzene gave a negative result: it did not exert any inhibitory action on the cultures.

Cytogenetic studies have been conducted on rat bone marrow cells following inhalation exposures to p-DCB (Anderson and Richardson, 1976). Three series of exposures were carried out: 1) one exposure at 299 or 682 ppm for two hours, 2) multiple exposures at 75 or 500 ppm, five hours/day for five days and 3) multiple exposures to 75 or 500 ppm, five hours/day, five days/week for three months. Benzene (at 10, 750 or 7,500 ppm) was used as the positive control in the first experiment. Vinyl chloride (at 1,500 ppm) was used as the positive control in the other two experiments. Negative controls breathed fresh air alone. In Experiment 1, three rats were in each treatment group, four in the negative control group. In the other two experiments, there were two rats in each treatment group and two rats in the negative control group. In Experiment 1, 50 cells from each animal were examined; in Experiments 2 and 3, 100 cells from each animal were examined.

Animals were sacrificed 22 hours after termination of exposure, following one hour after an intraperitoneal dose of colchicine. Bone marrow cells from both femurs were stained with Giemsa and surveyed for chromosome or chromatid gaps, chromatid breaks, fragments or other complex abnormalities. In all three experiments, exposure to p-DCB failed to induce any statistically significant effects indicative of chromosomal damage when compared to the negative controls, whereas in all experiments, the positive controls did produce damage at all levels employed. Also, there was no evidence of a dose-response relationship to p-DCB exposure, whereas, there was with benzene in Experiment 1. Thus, under the conditions of these experiments, p-DCB does not appear to elicit chromosomal damage.

Para-dichlorobenzene also was tested in a dominant lethal study in the CD-1 mouse (Anderson and Hodge, 1976). Fertile males were exposed, by inhalation, to plain air or to one of three levels of p-DCB, or, by other routes of exposure, to one of three substances known to produce dominant lethal effects in this assay system. Groups of males were subdivided into the following experimental groups. Numbers of animals in each group are shown in parentheses.

Group 1: Air (negative control)-(35)

Group 2: 75 ppm p-DCB, 6 hr/day for 5 days (16)

Group 3: 225 ppm p-DCB, 6 hr/day for 5 days (16)

Group 4: 450 ppm p-DCB, 6 hr/day for 5 days (16)

Group 5: 200 mg cyclophosphamide/kg, once by i.p.
injection on Day 5 (13)

Group 6: 150 mg ethyl methanesulfone/kg,
orally once a day for 5 days (5)

Group 7: 2.5 mg nitrogen mustard/kg,
once by i.p. injection on Day 5

The mating protocol consisted of placing two virgin females in a cage containing one fertile male. Five days later the females were removed to a separate cage. Two days later, the male was caged with two different virgin females. This process was repeated until the males had been mated at weekly intervals eight times. Females were sacrificed 13 days after the assumed date of fertilization, or 15-16 days after caging with the male. Uteri of these females were examined for live implants, early and late deaths. Statistically, the data were analyzed by a simple Chi-square and a week by week hierarchical analysis of variance.

Only one death occurred among males exposed to p-DCB. This was a male in the 75 ppm group during Week 3. The investigators suggested that the death was unrelated to p-DCB exposure. Five males in the cyclophosphamide positive control group died early, three during Week 6 and one each in Weeks 7 and 8.

Frequency of mating was affected in only two of the groups. At Week 7, in the 77 ppm-dosed males, the frequency of mating was 100%, but significantly fewer males mated with both females ($P < 0.05$).

The number and percentage of females becoming pregnant was decreased significantly at Weeks 6 and 7 among those mated to males exposed to 75 ppm ($P < 0.05$). The mean total of implants per pregnant female in each group also was determined. The ratio was significantly reduced in the 75 and 450 ppm groups at Week 8 when compared with the negative control. All positive controls showed significant reductions at Week 1 ($P < 0.01$), cyclophosphamide and nitrogen mustard at Week 2 ($P < 0.01$) and ethyl methanesulfone at Week 8 ($P < 0.05$) when compared with the negative controls.

Early fetal deaths were analysed in three ways. When determining the number of females with at least one early death, groups exposed to 225 ppm p-DCB showed an increase at Week 1 ($P < 0.05$). All positive controls showed significant differences at Weeks 1 and 2 ($P < 0.001$ or 0.05). When comparing the mean number of early fetal deaths/pregnancy, there were no significant differences in any of the p-DCB-exposed groups. All positive control groups exhibited differences in Week 1 and 2, with cyclophosphamide also in Week 3 ($P < 0.05$ or less).

Comparison of mean percentages of early deaths per total implants per pregnancy revealed significant differences in p-DCB treated groups in Week 6 in the 225 ppm group ($P < 0.05$). Values for the 75 ppm group in Week 1 were higher than the negative controls, but not significantly by Dunnett's "t" test. No significant differences were seen in late fetal deaths for any groups.

Utilizing the three methods of analysis, two indicated significant differences between p-DCB-exposed groups and negative controls. However, these changes were different at different times

and they did not occur in a dose-related manner. Therefore, the authors suggested that these changes were not biologically significant. In addition, they concluded that p-DCB, at least at the exposure levels tested, does not cause dominant lethal mutations in germ cells of CD-1 mice.

Carcinogenicity

Few studies have been reported which address the carcinogenic potential of the dichlorobenzenes. Some of these are inadequate for judging this characteristic.

A study by Parsons (1942) gave somewhat inconclusive results on the carcinogenic effects of p-DCB in mice. One group of mice was irradiated with an unspecified source of radiation, then given 0.2% intraperitoneal p-DCB in sesame oil in silica. One animal showed ascites and a sarcoma-like growth. This animal tumor, when grafted, gave 100% takes. In "control" mice, i.e., those that were not irradiated, one animal developed a sarcoma.

Murphy and Sturm (1943) studied the effects of p-dichlorobenzene on induced resistance to a transplanted leukemia in the rat. Forty rats were immunized by intraperitoneal injection of either defibrinated rat blood, or chopped 15 day old rat embryo. They were exposed to saturated p-dichlorobenzene vapors for two to three hours daily for 14 days, and then injected with 0.2 cc of leukemia cells.

Of the 40 animals in the immunized group exposed to p-dichlorobenzene, 67.5% had tumors. An immunized group not exposed to p-DCB recorded 20.5% tumors while the control (no immunization) was 84.2% positive. It would appear that p-dichlorobenzene modified the

induced resistance of the rats to the leukemia. However, there is not sufficient evidence to state that there are possible immunosuppressant effects resulting from exposure to p-dichlorobenzene.

In the two studies by Hollingsworth, et al. (1956, 1958) described earlier, the investigators did a cursory survey for tumor occurrence during the subchronic exposure to rats, rabbits and guinea pigs. No tumors were reported in any species after exposure to either substance (o- and p-DCB). Little confidence can be placed in these results as the conditions of study were inadequate for evaluating carcinogenic or mutagenic potential.

Guerin and Curzin (1961) found that dichlorobenzene (isomer not specified) gave a slight response for carcinogenic activity in mice, as measured by the sebaceous gland and hyperplasia tests. In both cases, dichlorobenzene (1 g/100 cc solution in acetone) was applied to the skin of Swiss mice three times (0.1 cc solution). The sebaceous gland test was based upon the disappearance of the glands after application of the test compound. The hyperplasia test examined the thickening of the skin epithelium after application. On an arbitrary scale of 0 to 4, (negative to strongly positive), dichlorobenzene scored 0.9 on the sebaceous gland test and 0.7 on the hyperplasia test.

The National Toxicology Program (NTP) recently completed carcinogenicity bioassays in two species of rodents (rat and mouse) for ortho- and para-dichlorobenzene. The results of the chronic gavage studies on the ortho isomer were presented to the NTP Board of Scientific Counselors in a draft report last year (NTP, 1982). However, the Board has not approved the report as yet. Nonetheless,

the results are summarized below. The chronic gavage studies on p-dichlorobenzene are complete. The results of these latter studies on p-DCB may be presented soon. The protocols for the chronic studies are presented below. In addition, the results of the studies conducted in support of the chronic experiments with both isomers also are discussed.

Two 14-day repeated dose studies with o-DCB were conducted in B6C3F1 mice as part of the prechronic test phase of the NTP bioassay on this substance (Battelle Columbus, 1978a, 1978b). These studies were designed to determine approximate doses for the three month subchronic toxicity study. No acute toxicity study was conducted. In the first study, gavage doses of 0, 250, 500, 1,000, 2,000 or 4,000 mg/kg in corn oil were administered daily to six groups of five mice of each sex (Battelle Columbus, 1978a). By the end of the study, 47 of the 60 mice had died, leaving 5 male and 8 female survivors. All mice in the top two dose groups died by Day 3. One female mouse in the 1,000 mg/kg group survived to the end of the experiment. The same was true for the 500 mg/kg group. One male and one female survived from the 250 mg/kg group. In addition, one male mouse in the control group died on Day 4.

At gross observation, early death mice had pale livers, beginning as early as Day 2 in the 4,000 mg/kg group and seen by Day 7 in the 250 mg/kg females. Other gross lesions observed in all dose groups included reddened lung areas and yellow-green or red small intestines. Liver histopathology in the six male mice receiving histopathological examination was characterized by moderate to severe centrilobular necrosis at the 500 mg/kg level

and milder (in one) or none (in two) at the 250 mg/kg level. At 500 mg/kg, one mouse exhibited lymphoid necrosis of the spleen, and the other animal, marked lymphoid depletion of the thymus. Of the seven females receiving histopathological examination, the one at 1,000 mg/kg surviving to the end of the study showed no pathology. Of the three examined from the 500 mg/kg group, one of two early fatalities exhibited no pathology while the other had moderate lymphoid depletion of the spleen. The one survivor had no lesions. At 250 mg/kg, one early fatality exhibited mild centrilobular hepatic necrosis, the other, no histopathology. The one survivor examined showed no pathology.

Since no no-effect dose level was determined from this initial experiment, it was decided that a follow-up study would be conducted, using a range of doses lower than that employed in the first experiment. The protocol was identical to that of the first study; however, the doses selected were 0, 30, 60, 125, 250 or 500 mg/kg in corn oil (Battelle Columbus, 1978b). In this study, only two early deaths occurred: one male in the 500 mg/kg group on Day 3 and one female in the 125 mg/kg group Day 8. Body weight changes in the treated animals were not markedly different from the controls, although males in the 30 and 60 mg/kg groups showed a 9% lag (a 6% increase vs. a 15.7% increase in the controls). During the first three days of the study, all mice in the highest dose group exhibited labored breathing, rough coats and watery eyes. The signs then disappeared. Gross examination was conducted on all animals. The male mouse dying on Day 3 had a pale, mottled liver, enlarged stomach and reddened small intestine.

Histological examination of livers from four males and four females from the 500 mg/kg dose group revealed no changes in two males, mild focal necrosis in one, and mild focal necrosis as well as cytomegaly, karyomegaly and chronic moderate multifocal granulomatous hepatitis in the fourth male. Among the four females, one exhibited moderate focal necrosis, another showed mild focal necrosis, and the other two had mild centrolobular degeneration with cyto- and karyomegaly. These changes were judged to be treatment-related. On the basis of the results of this second study, it was recommended that the subchronic study in the mouse be performed at the same dose levels used in this 14-day study.

The subchronic toxicity gavage study with o-DCB in mice was conducted to assist in dosage selection for the 104-week chronic study (Battelle Columbus, 1978c). The doses used were as detailed above (0, 30, 60, 125, 250 or 500 mg/kg/day). Treatment groups consisted of five animals of each sex. Single gavage doses of the compound in corn oil were administered 5 days/week for 13 weeks. Weekly individual body weights and cage food consumption rates were monitored. Animals were sacrificed on Day 92 or 93, with full necropsy, recording of organ weights and histological examination of various tissues. Special studies also were performed near or at the end of the exposure period. These included: urinalysis in the highest dose group and controls, hematology and clinical chemistry. Organ/body weight ratios were calculated and urine and liver porphyrin determinations made.

During the study, lethargy and rough coats were observed

in both sexes at the four highest doses. However, by the final week on test, only animals of both sexes at the 500 mg/kg dose and males at 250 mg/kg showed these signs. Body weight gain was affected in males at 500 and 250 mg/kg (-49% and -18%, respectively, when compared with controls). Female mice receiving 500 mg/kg/day exhibited a differential weight gain of -62% when compared with controls. No other groups showed differential weight gains of greater or less than 10% when compared with controls.

Hematological parameters evaluated included hemoglobin, hematocrit, total and differential white counts, red cell and platelet counts, mean corpuscular volume and reticulocyte counts. The investigators did not perform any statistical analyses on these data, but claimed that no clinically-significant treatment-related changes occurred. The apparent differences in white cell counts of treated males when compared with control was attributed to relatively low counts among the controls. It was suggested that these counts were below those typically observed in that laboratory and others ($3.4 \times 10^3/\text{mm}^3$ in controls vs. $5.4\text{--}6.6 \times 10^3/\text{mm}^3$ in the treated groups). Since the individual data were not available, the statistics cannot be done which would show whether or not there were significant differences between the controls and the treated groups. In addition, information on the "normal" counts is not available for evaluation. But, since there is at least anecdotal evidence of a possible relationship between exposure to ortho-dichlorobenzene and leukemias in humans, it would be prudent to evaluate these results in greater depth.

Blood samples were analyzed for alkaline phosphatase, SGPT and gamma-glutamyl-transpeptidase (GGTP). No GGTP was detected in any sample. Statistically significant dose-dependent changes in alkaline phosphatase did not occur, although increased levels were noted in males receiving 125 and 250 mg/kg/day. SGPT levels in the two surviving males receiving 500 mg/kg/day were increased significantly over control, due to the high value recorded in the animal exhibiting the hepatocellular necrosis. The other animal showed no hepatic histopathology.

The following parameters were monitored during the urinalysis: pH, glucose, protein, bilirubin, ketones, occult blood, specific gravity and creatinine. The report stated that the volume of urine collected from treated animals, especially the males, was generally greater than that collected from controls. The individual data were not available to corroborate this conclusion. No record of fluid intake was kept during the study. Decreases in specific gravity and creatinine were noted, reflecting dilution due to increased urine output. No other compound-related effects were observed.

Uroporphyrin levels in treated males were generally higher than in the controls. In females, coproporphyrin levels were higher in the treated animals than in controls. Coproporphyrin levels in treated males and uroporphyrin levels in females were not different from controls. Sex differences were seen in liver protoporphyrin levels, as a dose-dependent increase was observed in the females but not in the males.

Organs weighed were: heart, lung, kidney, testis, spleen, thymus, brain, ovary and uterus. While no statistics were done, it appeared that relative liver weights were increased in the highest dose males and females. An increase of lesser magnitude was seen in females receiving 250 mg/kg/day. No other differences were noted.

All of the above-mentioned tissues and skeletal muscle from control and highest dose animals were examined microscopically. The liver, thymus, heart, spleen and thigh muscle were examined from animals in the 250 mg/kg group and livers only from the 125 mg/kg group. Lesions were observed in all tissues from the highest dose animals. These were considered to be treatment-related, since they were absent or occurred less frequently in the controls.

Livers of the highest dose animals exhibited significant centrilobular necrosis, hepatocellular necrosis and degeneration, and deposition of yellow-green to golden pigment considered to be hemosiderin. The heart showed multiple foci of mineralization in the myocardial fibers. Skeletal muscle also exhibited mineralization as well as necrosis and myositis. Some animals had lymphoid depletion of the spleen and thymus. One female exhibited lymphocyte necrosis in the spleen.

Among the animals receiving 250 mg/kg, only hepatocellular necrosis was noted in two males, with pigment deposition in one male and hepatocellular degeneration in one male. No lesions were noted in the group treated with 125 mg/kg.

The results of this study suggest that an oral no-effect level can be identified in mice over 13-week exposure period of 125 mg/kg/day. Doses of 60 and 120 mg/kg/day were selected for use

in the 104-week chronic study.

A draft NTP Technical Report on the bioassay of ortho-DCB in mice and rats was presented to the NTP Board of Scientific Counselors on September 22, 1982. This document does not become a final report until it is reviewed and approved by the Technical Reports Review Subcommittee of the NTP Board of Scientific Counselors. Therefore, the text that follows represents only a preliminary assessment of the data from the bioassay.

The bioassay was conducted by administering 60 or 120 mg/kg doses of o-DCB in corn oil, five days/week for 104 weeks. Groups of 50 males and 50 females comprised each dosage group and a vehicle control group, as well. The control group received equivalent volumes of corn oil on the same schedule as the treated animals.

No difference in survival rates were observed between the treated and control groups of either sex. There was no evidence of compound-related nonneoplastic liver lesions, suggesting that a higher dose may have been tolerated in the chronic study.

Statistically significant positive trends in the incidence of malignant histiocytic lymphomas occurred in mice of both sexes ($P < 0.05$). However, the incidence of total lymphomas of all cell types was not significantly increased above control. The draft reports states that "since the histiocytic lymphoma is a controversial diagnosis among different pathologists and since all types of lymphomas have the same histiogenesis, an increase in this specific type of lymphoma in the absence of an increase in the total incidence of all types of lymphoma is not considered to be biologically significant."

An increase in alveolar/bronchiolar carcinomas were observed in male mice (control=4/5, 8% low dose=2/50, 4%, high dose=10/50, 20%). This increase was shown to be significant when analyzed by the Cochran-Armitage test, but not the life-table or incidental tumor test. Thus, this increase was discounted because the combined incidence of males with alveolar/bronchiolar adenomas or carcinomas was not significantly greater than controls by any of the three tests (control=8/50, 16%, low dose=8/50, 16%, high dose=13/50, 26%).

Male mice also exhibited a significant decrease in hepatocellular adenomas at the high dose (control=8/50, 16%, low dose=5/49, 10%, high dose=2/46, 4%). This decrease was accompanied by a negative dose-response trend using the Cochran-Armitage test. When total incidence of adenoma or carcinoma in the high dose males was evaluated, this decreased incidence was statistically significant only by the life table test (control=19/50, 38%, low dose=14/49, 29%, high dose=11/46, 24%).

The preliminary assessment suggests that, under the conditions of this bioassay, ortho-dichlorobenzene was not carcinogenic in the B6C3F1 mouse of either sex, but that the maximum tolerated dose was probably not achieved in the study.

A 14-day repeated dose study with o-DCB also was conducted in Fischer 344 rats as part of the prechronic test phase of the NTP bioassay on this substance (Battelle Columbus, 1978d). Single oral gavage doses of o-DCB in corn oil of 60, 125, 500 and 1,000 mg/kg were selected. Five animals of each sex were placed in one of five treatment or one control groups. All of 10 rats in the 1,000 mg/kg group died early, the males by Day 4 and the females by Day 5.

No other early deaths occurred. The percentage body weight gain decreased with increasing dosage in both sexes. Males at 250 mg/kg showed an -11% deficit, those in the 500 mg/kg group, a -16.6% differential. Only females at 500 mg/kg showed a weight gain deficit exceeding 10% (-11.8%).

No tissues from animals dying early were examined histologically. Those in the highest dose group exhibited, upon gross examination, pale and yellow livers, yellow and/or green or red colored contents in the small intestine, similarly-colored fluids in the urinary bladder, red fluid in the cecum and congestion of the vasculature of the brain. The liver lesion was interpreted to reflect hepatotoxicity. No toxic lesions were observed in other dosage groups during gross examination. Tissues from two males and two females receiving 500 mg/kg/day were examined microscopically, with no lesions indicating toxicity.

Based upon the results of this study, it was recommended that the 13-week subchronic gavage study in Fischer 344 rats employ doses of 25, 50, 100, 200 and 400 mg/kg/day, five days/week. The doses actually administered in the subchronic study were 30, 60, 125, 250 and 500 mg o-DCB in corn oil (Battelle Columbus, 1978i). Controls received corn oil. Special studies, as described for o-DCB in mice and p-DCB in both species, also were conducted in this study. No statistical analyses were performed on the data from these special studies, except for group means and standard deviations of those means.

Of the animals used in this study, only four died early: one male each in the 30 mg/kg (Week 11) and control (Week 10) groups and two females in the highest dose group (Weeks 6 and 9). Only nine males in the 250 mg/kg group completed the study; the tenth was found to be a female during Week 10 and was removed from the group.

Food consumption did not vary more than 10% in treated groups when compared with controls (down 1.5 mg/day in the highest dose males). Lack of body weight gain increased with increasing doses in both sexes. At 250 mg/kg and 500 mg/kg, this differential exceeded 10%. No striking differences in hematological parameters were noted, but without statistical analysis, it is difficult to determine if the changes are truly statistically non-significant. Those parameters which may have been altered significantly in the highest dose males were hematocrit (down 5%) and red cell count (down to $8.57 \pm 0.25 \times 10^6$ cells/mm³ from an average of $9.42 \pm 0.53 \times 10^6$ cells/mm³ in the control group). There appeared to be a trend in platelet count, directly proportional to increasing dose in the females, with levels of 300,000 in the controls to 365,000-600,000 as the dose increased, with the 250 mg/kg group falling out of sequence. This apparent change may have been due to what appeared to be a lower-than-normal count in the controls.

Of parameters measured in the clinical chemistry analyses, cholesterol levels were increased in males in 250 and 500 mg/kg and in females at 125, 250 and 500 mg/kg. Triglycerides dropped in high dose males. The combined alpha-globulin fraction appeared to be increased in males receiving 250 and 500 mg/kg and in females treated with 500 mg/kg. Of the parameters tested in the urinaly-

sis, urine volume was altered significantly. Output increased an average of 157% in treated males and 187% in treated females, when compared with their respective controls. Decrease in urine creatinine accompanied the dilution occurring with the increased volume output.

Porphyrin levels in the urine showed a striking increase in the highest dose animals (3-6 fold), both in coproporphyrin and uroporphyrin levels. Liver protoporphyrin levels were not altered significantly at any dose level. Thus, there appeared to be abnormal excretion of the porphyrin, but not retention.

Organ weights and organ/body weight ratios were determined. In rats of both sexes receiving 250 or 500 mg/kg, relative liver weights were increased. The liver/body weight ratios in the other treatment groups did not appear to differ from the controls. In addition, ratios for the other organs (spleen, kidney, testis, ovary, uterus, thymus, brain and heart) did not appear to have been altered.

No consistent lesions were noted upon gross examination at necropsy. Microscopic examinations were performed on tissues of all animals in the control and 500 mg/kg groups and on the thymus, liver and kidney of the animals from the 125 and 250 mg/kg groups. A moderate degree of centrilobular hepatocellular necrosis was seen in the livers of the highest dose rats which died early. Of the survivors in that group, most showed liver lesions, either centrilobular degeneration or necrosis of individual hepatocytes. This necrosis was characterized by randomly scattered cells that were pyknotic or karyolytic, with shrunken, dark red cytoplasm.

Some of the 500 mg/kg group males also exhibited renal tubular degeneration and lymphoid depletion of the thymus. These latter lesions were not seen in other treated groups or controls. However, hepatocellular necrosis was observed in the 250 mg/kg group and in one female treated with 125 mg/kg groups was considered to be hemosiderin since it was PAS- and Perls positive.

The liver lesions observed in the 250 and 500 mg/kg groups were considered to be dose- and treatment-related, and probably life-threatening, as were the renal and thymic lesions in the high dose group. The reviewing pathologist recommended that a Maximum Tolerated Dose (MTD) of 125 mg/kg (the apparent no-effect level) be set for both sexes of rats in the 104-week chronic study. Ultimately, doses of 60 and 120 mg/kg were chosen for this study.

As mentioned above, this assessment of the effects of o-DCB in the bioassay is preliminary, pending acceptance by the NTP Board of Scientific Counselors.

As with the mice, groups of 50 male and 50 female rats received 60 or 120 mg/kg doses of o-DCB in corn oil, five days/week for 104 weeks. Controls, also 50 of each sex, received an equal volume of corn oil on the same schedule.

There was no significant difference in the survival rates of female rats of either treatment group or low dose males when compared with the controls. However, there was a significant decrease in the survival of the males at the high dose ($P < 0.001$). However, several of the males dying before the end of the study were found to have amounts of corn oil or o-DCB in corn oil in their lungs (3 in the control group, 8 at the low dose and 12 at the high

dose). Therefore, gavage trauma may have contributed to their deaths. The report suggests that the lower survival rate among the dose males may not reflect that the maximum tolerated dose was exceeded. In fact, as was seen in the mice, there was no increase in the incidence of nonneoplastic lesions of the liver in rats receiving either dose of o-DCB, again suggesting that a higher dose might well have been tolerated.

The low dose males showed a significant increase in the incidence of adrenal pheochromocytomas when compared with the control group by life table analysis (control=9/50, 18%, low dose=16/50, 32%, high dose=6/49, 12%). The report concludes that since this incidence was significant only by one of the three tests, and this tumor expressed no dose-response trend or high dose effects, and there were no malignant pheochromocytomas, the increase seen in the low dose group was not related to treatment with o-DCB.

Interstitial cell tumors of the testis in the males also occurred with a significant positive trend when analyzed by the life table test (control=47/50, 94%, low dose=49/50, 98%, high dose=41/50, 82%), but with a significant negative trend when analyzed by the Cochran-Armitage test. The reports states that "since this tumor is not considered to be life-threatening, this increase detected by the life table test was discounted."

Preliminary assessment of these data suggests that, under the conditions of this bioassay, ortho-dichlorobenzene was not carcinogenic in the Fischer 344 rat of either sex, but that the maximum tolerated dose may not have been achieved.

Para-dichlorobenzene

A 14-day repeated dose oral gavage study was conducted in B6C3F1 mice to determine doses for the 13-week subchronic toxicity study (Battelle-Columbus, 1978e). Doses of 250, 500, 1,000, 2,000 or 4,000 mg/kg in corn oil were administered to groups of five males and five females. Controls received corn oil only on Day 1. All mice receiving 4,000 mg/kg died by Day 3. At 2,000 mg/kg, four males died by Day 7 and two females by Day 8. At 1,000 mg/kg, four males and two females died by Day 8. At 500 mg/kg, four males died by Day 8, but all females were dead by Day 5. At 250 mg/kg, three males died by Day 7, three females by Day 6. Among controls, two males and one female died on Day 3, presumably as a result of gavage trauma.

Upon gross necropsy, a number of lesions were noted in both sexes and at all dose levels. These included: livers of abnormal color, either yellow or tan or reddish-chocolate, soft or mushy small intestines from yellow-green through pink or red to black in color, and pink to bright red lungs. Occasionally, kidneys were pale, and there occurred enlarged, chalk-white mandibular salivary glands. According to the investigators, no lesions were seen microscopically in either sex or at any dose level which indicated significant toxicity.

Since the study did not establish a no-effect level, a second 14-day repeated dose study was performed (Battelle Columbus, 1978g). Dose levels of 60, 125, 250, 500 and 1,000 mg/kg in corn oil were employed. The controls were untreated. Only one early death, a male in the 125 mg/kg group, occurred, purportedly due to gavage trauma. Only the 500 mg/kg females showed a decreased body weight

gain (-10.9% vs. control). No gross pathology was noted at necropsy. No histology was performed.

On the basis of these two 14-day studies, the principal investigators recommended that the subchronic gavage studies in mice be performed at doses of 125, 250, 500, 1,000 and 2,000 mg/kg/day, 5 days/week, for 13 weeks. However, the final doses actually employed in the first subchronic study were 600, 900, 1,000, 1,500 and 1,800 mg/kg/day (Battelle Columbus, 1979a). Ten males and ten females were assigned to each group. Special studies included: urine and liver porphyrin determination, calculation of organ/body weight ratios, urinalysis, clinical chemistry and hematology.

Twenty-six animals (12 males and 14 females) died before the end of the study. Seven males and females in the 1,800 mg/kg died early, most within the first week. At 1,500 mg/kg, three males and five females died early. One male in the 1,000 mg/kg group and one control male died during the last week.

No effects on food consumption were noted in any group. Differential body weight gains in treated males of all groups were significantly different from controls. These differences ranged from -50% at 1,500 mg/kg to -22.9% at 1,000 mg/kg. The differences did not show a dose-related trend. Females showed a differential weight gain of -39% at 600 mg/kg, with lesser changes as the dose increased, but still > 10%. Since food consumption was not altered, the weight gain reductions were apparently unrelated to dietary intake.

The hematological parameters included: hemoglobin, hematocrit, total and differential white cell counts, red cell counts, mean corpuscular volume, platelet and reticulocyte counts. No

statistical analyses were performed on these data. However, the investigators stated that there appeared to be no significant differences between treated groups and controls. However, a cursory review of the group data suggest that there may have been a significant decrease in platelet counts in the high dose males ($445,000 \pm 8,700$ vs $691,900 \pm 209,200$ for controls), and a significant increase in surviving females at the two highest doses ($707,400 \pm 41,000$ at 1,500 mg/kg, $707,500$ at 1,800 mg/kg vs $492,000 \pm 86,631$ for controls). This latter observation may be the result of a lower than normal platelet count among the control animals.

The following analyses were performed on blood samples drawn at the time of sacrifice: serum glutamic pyruvic transaminase (SGPT), alkaline phosphatase, gamma-glutamyltranspeptidase (GGTP), bilirubin, cholesterol, triglycerides, blood urea nitrogen (BUN), glucose and total protein. While no statistical analyses were performed on the data, it appeared that triglycerides were increased in males at the two highest dose levels, and that cholesterol was increased in all males except those in the 600 mg/kg group. Total protein appeared to be increased in males in the 1,800 mg/kg group. Since there were no increases in hemoglobin or hematocrit, this increase likely was due to an actual, rather than a relative, increase in blood proteins.

Both SGPT and bilirubin may have been increased in females in the two highest dose groups. No control values in females were recorded for cholesterol, triglycerides, BUN, glucose or total proteins. Thus, it is difficult to speculate whether or not the increase in cholesterol values in these groups was significantly

greater than control, as would be suggested by the dose-related upward trend, the relationship observed in the males.

During urinalysis, the parameters measured were: pH, protein, glucose, ketones, bilirubin, occult blood, specific gravity and creatinine, as well as uroporphyrins and coproporphyrins in the controls and two highest dose groups.

Ketonuria was noted in one of two pooled groups of males at the 1,500 mg/kg dose level and in both pooled groups of females at that dose. None was observed in urine of animals at other doses or in the controls. In the males, this occurrence may have been due to the severe body weight gain differential, according to the investigators. However, this postulate does not hold up for the females, since this severe weight gain differential was not observed. The investigators suggested that this observation was due to contamination of the urine samples. As was seen in the o-DCB studies, increased urinary output occurred, especially in the males.

Urinary coproporphyrin levels in both sexes appeared to increase significantly at the 1,500 mg/kg/dose level but only in females at the highest dose. No change was seen in coproporphyrin levels in males, but appeared to be lower in the surviving female at the highest dose. Dose-dependent increases in liver protoporphyrin in both males and females occurred. However, since no statistics were applied to the data, one cannot identify the lowest dose level at which significant increases occurred.

Of the organ/body weight ratios calculated, those for the liver were increased at all dose levels for both males and females. In addition, relative uterine weights of females decreased in a dose-related manner, with the greatest decrease in the ratio observed in mice receiving 1,800 mg/kg. No differences were apparent for lung, heart, kidney, spleen, thymus, brain, testis or ovary.

The only lesions noted during necropsy were some pale and/or yellow livers and pale kidneys in the highest dose group. No hemosiderin was noted in any liver or kidney of mice from any dosage group.

A number of lesions were noted upon histological examination. These included lesions of the spleen, thymus, bone marrow and lymph nodes among animals from the two highest dose groups. These were considered to be treatment-related. Hepatocellular changes which were seen in treated animals of all groups were not seen in the controls. In mice from the 1,500 mg/kg group which died before the end of the study, but not those who survived to the end, there was lymphoid depletion of the spleen, lymphoid necrosis in the thymus and hematopoietic hypoplasia in the spleen and bone marrow. The hepatocellular changes observed in the various groups included karyomegaly, cytomegaly, and occasionally, large prominent nuclei with variations in their shape along with changes in the number of centrolobular hepatocytes. The cytoplasm of these enlarged cells was grainy, sometimes hazy and amphophilic. These changes were dose-related.

Since the hepatocellular changes were seen in animals from all treated dose groups, no no-effect level could be established from the study results. The changes observed at 600 mg/kg could be characterized as minimal. Subsequently, a second subchronic study was performed in the attempt to establish the maximum tolerated dose for the chronic study and a no-effect dosage level for subchronic exposure (Battelle-Columbus, 1980a). In this second study, target doses of 75, 150, 300, 600 or 900 mg/kg/day were chosen. As shown later, because of an error in the preparation of the doses, those actually administered were 84.4, 168.8, 337.5, 675 or 900 mg/kg/day, five days/week for 13 weeks. The protocol was otherwise identical to the first subchronic study, except that no special studies were performed. During the study, three males and seven females died early. These deaths were not dose-related, but rather attributable to gavage trauma. No significant differences were apparent in dietary consumption or relative weight gains between treated and control animals of either sex.

During gross necropsy, no consistent observations were made which were considered significant. Microscopically, there was a significant number of animals in the two highest dose groups exhibiting centrilobular to midzonal hepatocytomegaly. Few changes of this type were seen in animals treated at 337.5 mg/kg/day. The investigators determined that 337.5 mg/kg/day was the maximum tolerated dose for this duration of exposure.

(104-week results to be added later)

Fourteen-day repeated dose and 13-week subchronic gavage studies also were conducted in Fischer 344 rats as dose-range finding efforts preliminary to the 104-week chronic bioassay for p-DCB in this species. The first repeated dose study employed single doses of 60, 125, 250, 500, or 1,000 mg/kg/day p-DCB in corn oil (Battelle Columbus, 1978f). Doses were administered to five males and five females per group. Controls were untreated.

Only one early death was recorded, a male at the 125 mg/kg dose level on Day 8. Body weight gains were slightly suppressed in the males, with the greatest difference being seen at the highest dose (-9.2%). No gross or histological pathology was observed. Because of the absence of significant signs of toxicity, it was decided that the study be repeated at higher dose levels.

A re-run of the 14-day repeated dose study was conducted according to the protocol of the first study. In this study, however, doses of 500, 1,000, 2,000, 4,000 or 8,000 mg/kg/day were administered in corn oil to the rats (Battelle Columbus, 1978g). Again, controls were untreated. No clinical pathology studies or histological examinations were done.

By Day 3, all animals in the group receiving 2,000, 4,000 or 8,000 mg/kg had died. Gross pathology included pale livers, discolored lungs and intestines and mandibular lymph nodes and fluid discharge from the eyes and nose. One male rat receiving 1,000 mg/kg died on the first day, apparently due to gavage trauma. Four females in that dose group died on Day 1 (1), Day 4 (1) and Day 5 (2), with those dying on Day 5 showing slightly congested livers. One female receiving 500 mg/kg died on Day 13 of gavage trauma.

At these higher doses, evidence of depressed body weight gain appeared. Among males, those receiving 1,000 mg/kg/day had a 22% reduced gain; those receiving 500 mg/kg/day showed a 24% reduced gain. No significant differences were seen in the females at any dose.

From the results of the two repeated dose studies it was determined initially that a subchronic gavage study in the Fischer 344 rat be performed at dose levels of 60, 125, 250, 500 or 1,000 mg/kg/day. In fact, the first subchronic study utilized doses of 300, 600, 900, 1,200 or 1,500 mg/kg/day (Battelle Columbus, 1979b). The protocol included use of ten animals/sex/dose or control group. Animals received doses of p-DCB in corn oil, five days/week for 13 weeks. Vehicle controls received corn oil alone. In addition to gross necropsy and histological examination, additional special studies were performed as described for the subchronic studies on o-DCB. These included: clinical chemistry and hematology on blood samples drawn the day of sacrifice, urinalysis on animals at the 1,500 and 1,200 mg/kg groups and controls, porphyrin analysis in urine and liver of the same groups and calculations of organ/body weight ratios. Again, no statistical analysis of these data was performed.

Eight early deaths occurred in the 1,500 mg/kg males, and five in males at 1,200 mg/kg. One male receiving 900 mg/kg died during Week 1, and one control male during Week 10. Among females, 9 of 10 receiving 1,500 mg/kg died early, one of 10 at 1,200 mg/kg and two of 10 at 900 mg/kg. The males tended to die earlier than the females.

Food consumption in the treated groups varied little from control. The one remaining male at 1,500 mg/kg showed a slight increase compared with controls. Male rats at 300 mg/kg averaged slightly less than controls. A dose-dependent depression of body weight gain was seen in both sexes. In females, a greater than 10% difference was seen at the 900 mg/kg level and above. All treated males exceeded the 10% differential when compared with controls.

Of the hematological parameters analysed (hemoglobin, hematocrit, mean corpuscular volume, red cell, reticulocyte and platelet counts, total and differential white cell counts), the hemoglobin and hematocrit levels appeared to be lower in the two surviving males at 1,500 mg/kg. Hemoglobin levels were 17.6 ± 0.6 G/dl in controls vs. 15.3 ± 0.2 G/dl in the treated rats. These two males also exhibited mild anemia with average red cell counts of $8.85 \pm 0.22 \times 10^6$ cells/mm³ compared with control levels of $10.03 \pm 0.36 \times 10^6$ cells/mm³. Mean corpuscular volume also was decreased slightly (51 ± 2 in controls vs. 48 ± 0 in the treated rats). The investigators reported that no histological evidence of bone marrow changes were apparent in these two rats, although one other male and five females treated with this dose did exhibit bone marrow hypoplasia. The single surviving high dose female showed an increase in hemoglobin, lowered white cell count, increased red cell count and decreased mean corpuscular volume when compared with controls. However, one cannot establish the significance of these observations, since they occurred in a single animal.

Clinical chemistry analyses on blood drawn at sacrifice included: gamma-glutamyl-transpeptidase, alkaline phosphatase, bilirubin, cholesterol, triglycerides, total protein, blood urea nitrogen, glucose and globulin fractions. Of these parameters, there was evidence of a possible dose-related increase in alkaline phosphatase in females, with a substantial increase in the lone survivor at the highest dose. Trends also appeared in both sexes as an increase in cholesterol levels with increasing dose and an increase in albumin levels at the highest doses. The total protein increases seen at the higher doses likely are due to the increase in the albumin levels.

The investigators reported no significant changes in the parameters measured in the urinalysis: pH, protein, glucose, creatinine, ketones, bilirubin, occult blood and specific gravity. As previously described for o-DCB, urine volume of rats treated at the higher dose levels was significantly increased: 246% in males at 1,200 mg/kg, 142% in females at this dose. The few survivors from the 1,500 mg/kg groups had urine volumes 6-10 times greater than control levels. Since drinking water intake was not monitored, one cannot speculate on the consequences of this alteration in output.

Porphyrin levels in urine and liver also were monitored in the two higher dose group and control males. Significant increases in both coproporphyrin and uroporphyrin levels in the urine were observed. A similar increase was seen in the urine of the surviving highest dose female. No data for survivors at 1,200 mg/kg were presented. Liver protoporphyrin levels apparently were

not increased in either sex. On the contrary, the levels in the survivors at the highest dose were depressed somewhat. Therefore, there was no retention of porphyrins in treated animals, just as was reported for o-DCB.

Several organs were weighed at the time of sacrifice. These included: heart, liver, kidney, uterus, ovary, testis, brain, thymus and spleen. The relative liver weights were increased clearly in animals of both sexes at all doses except the lowest (300 mg/kg). An increase at this dose level was seen in females, but it may not have been statistically significant. Dose-related changes in brain/ and uterus/body weight ratios also were observed. The investigators suggested that these differences might be due to the significant lack of body weight gain in the affected groups at the higher dose levels.

Histological examination was performed on tissues from animals in the control and three highest dose groups. The kidneys and lungs from males treated at 300 and 600 mg/kg also were examined.

Retinal atrophy was seen in almost all of the animals, the degree of severity of which was inversely related to the time of death. Thus, those dying early showed few signs; those dying later had severe bilateral atrophy.

Pulmonary lesions related to the gavage procedure were seen in animals of all tested groups. Presumably, aspiration of the test material occurred.

Of significance was the degeneration and necrosis of the hepatocytes, hypoplasia of bone marrow, lymphoid depletion of the spleen and thymus, epithelial necrosis and villar bridging of the

mucosa of the small intestine and epithelial necrosis of the nasal turbinates that occurred in animals in the two highest dose groups. None of the lesions were observed in animals receiving doses of 900 mg/kg or less. Thus, these changes were considered to be treatment- and dose-related.

Most of the males surviving beyond the halfway point of the study exhibited renal lesions characterized by multifocal degeneration or necrosis of the cortical tubular epithelium. An amorphous eosinophilic material was often present in the lumen of these tubules. Some thickening of the basement membrane of these cells was visible. Occasionally, a dilatation of some tubules could be observed at the corticomedullary junction. These tubules also had degenerated epithelia and were filled with material similar to that described above.

The investigator describing the pathology stated that the renal lesions are not unusual for male rats of this strain. However, he would have expected to have seen lesions of some magnitude in the control animals, and to a lesser degree in treated ones. Thus, he concluded that while the lesion was not clearly dose-related, it might be at least partially treatment-related and for this reason, no no-effect dose level could be established from the results of this study. Thus, it was decided that the subchronic gavage study in rats would be repeated, employing lower dose levels of 37.5, 75, 150, 300 or 600 mg/kg/day (Battelle Columbus, 1980b). The protocol employed was identical to that described above for the first subchronic study, except that no special studies were done. A few early deaths occurred during the second study. All were attributed to gavage trauma.

No significant differences in food consumption were observed. No dose group had a greater than -10% differential weight gain when compared with controls. Histological examination was performed on all control and 600 mg/kg dose animals, as well as all early death animals, and kidney from males at the two lower doses.

Microscopically, some lung pathology was noted which was attributed to aspiration of the gavaged material. There also was an increased incidence and severity of renal cortical degeneration in males receiving the two higher doses, as had been observed in the earlier study. Females showed no significant changes at any dose level. Some rats also exhibited myocardial degeneration and lymphoid hypoplasia in lung tissue. These changes were characterized as being normal for this strain and age of rat. The reviewing pathologist suggested that the MTD for males be set at 150 mg/kg and for females at 600 mg/kg for the chronic study. Therefore, Battelle Columbus proposed doses for the chronic study of 75 and 150 mg/kg in males, 300 and 600 mg/kg in females. Tracor Jitco suggested 150 and 300 in males, 300 and 600 mg/kg in females.

(104-week study to be added later.)

Other Carcinogenicity Studies

Long term inhalation studies with p-DCB have been conducted in mice and rats. Groups of Swiss strain mice (75 males and 75 females per group) were exposed to airborne concentrations of 0, 75 or 500 ppm p-DCB five hours/day, five days/week for 57 weeks for all female groups and the 500 ppm males and for 61 weeks for the 0 and

75 ppm males. Males were sacrificed at these times. Females were held unexposed until sacrifice after 75 or 76 weeks.

The original objective of this study was to assess the chronic toxicity and carcinogenic potential of p-DCB in the mouse. However, because of fighting among the males during the early stages of the study and a high background incidence of respiratory disease in both sexes resulting in high mortality rates, the investigators felt that this objective was not attained satisfactorily. The following information was gathered from the study, however.

At termination, blood samples were drawn from at least ten males in each group to determine the blood levels of urea and glucose and serum alanine and aspartate transaminase activities. Because of insufficient or clotted samples, no urea or glucose levels were obtained from the 500 ppm group. No significant changes in blood glucose or plasma alanine transaminase activity were observed in the 75 ppm group. Blood urea concentrations appeared to be slightly reduced in the 75 ppm group, but this likely was due to the relatively high control levels observed. There was some evidence of an increase in the plasma aspartate transaminase activities in both treatment groups, but this increase was not statistically significant.

Urinalysis was performed for pH, glucose, bilirubin, specific gravity, protein and coproporphyrin, in males only. No significant differences were observed in any of the parameters. Several hematological parameters were studied in the males: hemoglobin levels, packed cell volume, total white cell count and differential, platelet count and red cell morphology, red cell count, mean red

cell count, mean hemoglobin concentration and methemoglobin content. Femoral bone marrow smears also were examined. Slight reductions in hemoglobin concentration and packed cell volume were observed in isolated males in all three groups. These animals also showed slight increases in methemoglobin. Among the 500 ppm-dosed animals, there was a slight, but not statistically significant, decrease in the mean total white cell count. No bone marrow changes were observed.

While tissues from animals of both sexes and all treatment groups were examined grossly, only those from females sacrificed when moribund or at the end of the study were examined histologically. The "epithelial repair" observed in the nasal sinuses and the "resolving pneumonia" seen in the lungs of both test and control animals were said to be related to the high incidence of respiratory disease in the colony, thought to be caused by a Sendai virus. Lesions in the liver (hepatitis) and the kidney (inflammation) were seen in similar quantities in both control and test animals. No significant increases in numbers of neoplastic lesions were observed in either treatment group when compared with the control group run concurrently or with historical controls from this laboratory.

From the data available, it was concluded that the administration of p-DCB by inhalation at levels up to 500 ppm for longer periods of exposure, followed by a period of recovery, did not produce any significant non-neoplastic lesions or increase the number or types of neoplastic lesions in female mice.

A similar long term inhalation study was conducted in Alderly Park Wistar-derived albino rats (Riley, et al., 1980a). Groups of animals (76-79 animals/sex/group) were exposed to airborne concentrations of 0, 75 or 500 ppm p-DCB five hours/day, five days/week for 76 weeks. Survivors at this time were left unexposed for 36 additional weeks. Interim sacrifices (5 animals/sex/group) were conducted at 26, 52 and 76 weeks. Body weights were determined weekly until Week 13 and then monthly thereafter. Food and water consumption was monitored biweekly until Week 15, then every six weeks thereafter. Urinalysis, clinical chemistry and histopathological parameters as described above for the mouse study also were analyzed in the rat study. Hepatic aminopyrine demethylase activity also was measured at the 52 week sacrifice. Organ weights were measured at each sacrifice date.

No consistently significant effects on mortality rates, food and water consumption, body weight gain, clinical chemistry, hematology or histopathology were observed in either sex, at either exposure dose or at any of the sacrifice times when compared with controls at the same times. Group mean body weights in the high dose females were significantly depressed from Weeks 4-38 except at Week 10, but were not significantly different at Week 50 or later. Increase in liver and kidney weights (both sexes at Weeks 76 and 112), heart and lung (both sexes at Week 112) and urinary protein and coproporphyrin output (in males) were noted in animals exposed at 500 ppm. No increases in tumor incidence or types were produced at either dose in either sex. The investigators concluded that, under the conditions of this study, p-DCB was not

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carcinogenic to rats at doses up to 500 ppm. An increase in hepatic hyperplasia was seen in treated females, but not in controls or treated males. Hemosiderosis was increased in treated males at both doses, but not in females. Focal chronic hepatitis with infiltration was increased in all treated animals, but significantly so only in the animals exposed at 500 ppm. A slight increase in myocardial calcification was seen in high dose males. Adrenal hyperplasia was increased only in the low dose males. The investigators concluded that non-neoplastic changes of a minor nature occurred in the high dose animals, but were absent or insignificant in the low dose animals.

VI. HEALTH EFFECTS IN HUMANS

Most of the poisoning incidents reported in the literature resulted from inhalation. Accidental inhalation can occur either at home or at work. There are several cases, however, when a chlorinated benzene was accidentally or deliberately ingested. Table VI-1 summarizes the available literature for ortho- and para-dichlorobenzene. Again, no reports appear in the literature concerning m-DCB.

o-Dichlorobenzene

One case of sensitization to o-dichlorobenzene was reported for a man who regularly handled window sashes dipped in the compound (Downing, 1939). When applied to the skin, there was a burning sensation after 15 minutes and for the duration of exposure. The site of application showed a reddish hue which increased up to 24 hours later, when blisters formed. A brown pigmentation formed later and persisted for three months. The man was forced to seek alternative employment.

Dupont (1938) described the reactions of a group of sewage workers performing cleaning operations in the sewer at a point directly below a pipe discharging sewage from a dry-cleaning establishment. Inhalation of the fumes caused irritation of the eyes and upper respiratory tract and vomiting. Apparently, no deaths occurred, but no clinical follow-up was described.

An 18-year old female employee of a dry-cleaning shop was admitted to the hospital with severe acute anemia (Gadtrat, et al., 1962). She had been employed as an ironer in that shop for about 6 months prior to admission. During her employment, she was

Table VI-1

Report of Human Exposure to o- and p-Dichlorobenzene
(Modified from Ware, West, 1977)

Compound	Subject	Exposure	Symptoms	Clinical Report	Follow-up Studies	Reference
o-DCB	Sewage workers	o-DCB effluents from dry-cleaning establishment above sewer	Eye and upper respiratory tract irritation, vomiting	o-DCB intoxication	Not indicated	Dupont, 1938
	47 year old male	o-DCB in dipping solution for window sashes, occupational	Water blisters on face, hands, arms	Eczenatoid dermatitis due to o-DCB	Not indicated	Downing, 1939
	18 year old female	Dry-cleaning and dyeing shop	Pallor, tiredness, headaches, vomiting, violent gastric pains	Severe hemolytic anemia: 1.5×10^6 erythrocytes/mm ³	10 months later erythrocytes "excellent" but leucocyte equilibrium showed tendency to neutropenia	Gadrat et al., 1962
	40 year old male	1940-1950 occupational exposure to solvent: 80% o-DCB, 15% p-DCB	Weakness, fatigue	Chronic lymphoid leukemia	Treatment ongoing	Girard, et al., 1969
	53 year old male	1932-1961, glue containing 2% o-DCB, methyl ethyl ketone, cyclohexane, (no benzene or homologues)	Weakness, fatigue	Chronic lymphoid leukemia, peripheral and abdominal adenopathy, splenomegaly	Died 1968	Girard et al., 1969

Table VI-1 (Continued)

Compound	Subject	Exposure	Symptoms	Clinical Report	Follow-up Studies	Reference
o-DCB	15 year old female	Cleaned clothes with products containing 37% o-DCB, (no benzene or toluene)	Initially hospitalized with retroclavicular adenopathy	Acute myeloblastic leukemia	Died 10 months later of 100% peripheral leukoblastosis	Girard et al., 1969
	60 year old male	1930 to 1960 shipping mono, o-di- and tri-chlorobenzene	Weakness, tiredness	Anemia: 3×10^6 erythrocytes/mm ³	Not indicated	Girard et al., 1969
p-DCB	62 year old male	p-DCB in bathroom	Asthenia, dizziness	Light hyperchromic anemia, after 1 month, increase in anemia, hypogranulocytosis	General hematological improvement but increase in hypogranulocytosis at 6 months	Perrin, 1941
	19 year old female	Preparation of p-DCB for 18 months	Asthenia, dizziness, weight loss	Slight anemia, reactional hyperleucocytosis	Not indicated	Petit and Champeix, 1948
	60 year old male	Heavy p-DCB moth ball vapor in house for 3 to 4 months	Weight loss, loose bowels, tarry stools, numbness, clumsiness	Acute yellow atrophy of the liver (confirmed by autopsy)	Developed ascites and died	Cotter, 1953
	Wife of male above	As above	Weight and strength loss, abdominal swelling, jaundice	Acute yellow atrophy of the liver (confirmed by autopsy), splenomegaly	Died 1 year after initial exposure	Cotter, 1953
	36 year old female	p-DCB moth killer in house	Periorbital swelling, intense headaches, profuse rhinitis	Exposure to p-DCB	Symptoms subsided within 24 hours	Cotter, 1953

Table VI-1 (Continued)

Compound	Subject	Exposure	Symptoms	Clinical Report	Follow-up Studies	Reference
p-DCB	34 year old female	Demonstrating p-DCB containing products	Tiredness, nausea headache, vomiting	Subacute yellow atrophy and cirrhosis of the liver	Not indicated	Cotter, 1953
	52 year old male	p-DCB exposure in fur storage plant	Weakness, nausea, blood, vomiting, jaundice.	Subacute yellow atrophy of the liver	4 years later reported "in good health"	Cotter, 1953
	20 year old male (+ 26 workmates)	p-DCB manufacture 1 to 7 months exposure	Loss of weight exhaustion, decrease of appetite	Methemoglobinemia and other blood pathologies	All workers transferred to other working environment	Wallgren, 1953
	53 year old female	12 to 15 year exposure to p-DCB moth balls in house	Cough, progressive dyspnea, fatigue, mucoid sputum	Pulmonary granulomatosis? focal necrosis of liver	Not indicated	Weller and Crellin, 1953
	3 year old male	Played with p-DCB crystals	Cough, listlessness, black urine	Acute hemolytic anemia	Complete recovery	Hallowell, 1959
	19 year old female	4-5 p-DCB pellets ingested daily for 2-1/2 years	Increased patchy pigmentation of skin	Due to p-DCB ingestion. Unsteadiness and tremors on ceasing consumption thought to be psychological, not physiological	Pigment returned to normal	Frank and Cohen, 1961
	21 year old pregnant female	Pica for p-DCB toilet blocks first trimester	General tiredness, mild anorexia, dizziness, edema of ankles	Hemolytic anemia	Healthy child delivered several months later	Campbell and Davidson, 1970

continuously exposed to fumes of cleaning solution containing 95% o-DCB and 5% p-DCB. She exhibited pallor, weakness, headaches, vomiting and severe pains. She was diagnosed as having a severe hemolytic anemia ($1,500,000$ RBCs/mm³), accompanied by leukocytosis and polynucleosis, and the presence of some immature elements belonging to the granulocytic and erythrocytic series. Vigorous treatment and a change of employment resulted in essentially a full recovery.

Girard, et al. (1969) reported three cases of leukemia which they attributed to chronic exposure to o-dichlorobenzene (o-DCB). One man hospitalized for chronic lymphoid leukemia worked with a solvent containing 80% o-DCB and 15% p-DCB for 10 years. A girl hospitalized with acute myeloblastic leukemia died 10 months later of peripheral leukoblastosis. She reportedly had a neurotic compulsion to remove dirt and grease stains from her clothes, which she did repeatedly with a product containing 37% o-DCB (no benzene or toluene). Another man exposed to a glue containing 2% o-dichlorobenzene, methyl ethyl ketone and cyclohexane for a period of 29 years died of chronic lymphoid leukemia. No further details of these incidents were given.

Girard, et al. (1969) also reported the case of a 60-year old male, who for 30 years had worked in a job during which he had been in contact with mono-, o-di- and trichlorobenzene. At the time he was seen by the authors, he exhibited symptoms of weakness and tiredness. Clinical studies revealed that he suffered from anemia, with an erythrocyte count of 3 million/mm³. No follow-up of this individual was described.

In cases where moderate exposures to p-dichlorobenzene were documented, patients complained of severe headaches, profuse rhinitis and periorbital swelling for approximately 24 hours after exposure (Cotter, 1953; Campbell and Davidson, 1970). Anorexia, nausea, vomiting, weight loss and yellow atrophy of the liver were reported for high exposure concentrations (Petit and Champeix, 1948; Cotter, 1953; Hallowell, 1959).

Wallgren (1953) reported loss of weight, exhaustion, decrease of appetite and blood dyscrasias in 27 men who manufactured p-dichlorobenzene for 1 to 7 months. Cotter (1953) described the case of a woman who demonstrated products containing p-DCB and who complained of tiredness, nausea, headache and vomiting. Clinical studies showed that she had subacute yellow atrophy and cirrhosis of the liver.

Heavy use of p-dichlorobenzene as either a moth-repellent or a deodorizer apparently resulted in weakness, nausea, vomiting of blood and jaundice (Perrin, 1941; Cotter, 1953; Weller and Crellin, 1953). One man and his wife died within months of each other of acute yellow atrophy of the liver (confirmed by autopsy). Their house was apparently saturated with p-DCB moth ball vapor for a period of at least three to four months (Cotter, 1953).

There are at least two reports of deliberate ingestion of p-dichlorobenzene. One woman who developed a pica for p-DCB during the first trimester of her pregnancy complained of general tiredness, mild anorexia, dizziness and edema of the ankles. She was hospitalized with hemolytic anemia and delivered a healthy child several months later (Campbell and Davidson, 1970). Another

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woman who ingested 4 to 5 p-DCB pellets (size not indicated) daily for 2 1/2 years complained about increased patchy pigmentation. Unsteadiness and tremors occurred when she stopped taking the pellets, but these symptoms were thought to be due to psychological rather than physiological withdrawal (Frank and Cohen, 1961).

VII. MECHANISM(S) OF TOXICITY

Of the various toxic effects occurring after exposures to the dichlorobenzenes, information on the mechanisms of toxicity is available only for the necrosis noted in the liver, which perhaps also can be applied to similar changes in the kidney and lung, and for the induction of porphyria via acceleration of synthesis in the heme pathway.

Many workers have studied the possibility that cellular damage caused by many drugs and xenobiotics is mediated via chemically reactive metabolites. Many of the metabolites formed are chemically inactive, but certain of the metabolites such as the arene oxides or epoxides may interact with physiological or biochemical processes, causing either pharmacological or toxicological effects.

Studies of halogenated benzenes, including the dichlorobenzenes, demonstrate that hepatic necrosis produced on exposure to these compounds results from their conversion to reactive toxic intermediates. Reid and co-workers have shown that an increase in toxicity can be correlated with an increase in covalent binding of metabolites to proteins within liver cells. This relationship can be seen both in vivo and in vitro. In the presence of the resulting hepatic necrosis, an increase in mercapturic acid excretion can be measured, as well as a decrease in available glutathione levels (see Table VII-1). This suggests that with sufficient depletion of glutathione (greater than 20-25%), the liver loses its ability to "detoxify" the chemical by complexing with the substance to form a less reactive substance, and, thus, proportionately more reactive

Table VII-1

Covalent Binding, Hepatotoxicity and Mercapturic Acid Excretion of Halogenated Benzene Derivatives

(Reid et al., 1971; Reid et al., 1973; Reid and Krishna, 1973)
(After Ware and West, 1977)

Compound	Covalent binding (nM/mg protein + S.E.) ($\bar{N}=6$)	Hepatic necrosis	Mercapturic acid excretion	Glutathione concentration (% of control)*
Monobromobenzene (1 mM/kg)	0.534 ± 0.050+	Yes	3 +	67
Monochlorobenzene (1 mM/kg)	0.604 ± 0.044+	Yes	3 +	Not determined
Moniodobenzene (1 mM/kg)	0.323 ± 0.054	Yes	3 +	66†
Monofluorobenzene (1 mM/kg)	0.060 ± 0.004+	No	+	82
o-Dichlorobenzene (0.5 mM/kg)	0.234 ± 0.015\$	Yes	2 +	48†
p-Dichlorobenzene (0.5 mM/kg)	0.021 ± 0.002\$	No	+	101

* Glutathione concentration determined 3 hours after administration of the hydrocarbon.

+ Killed at 24 hours

† $P < 0.01$ compared with control.

\$ Killed at 6 hours.

metabolite is available to interact with tissue proteins, resulting in cellular and tissue damage. A threshold does seem to exist for the halobenzene-induced necrosis. (Reid, et al., 1971; Reid, et al., 1973; Reid and Krishna, 1973). The stimulation of metabolism by pretreatment with phenobarbital potentiates hepatic damage in rats, as can be seen in Table VII-2. Conversely, blocking metabolism by SKF-525A (2-diethylaminoethyl-2,2-diphenylvalerate hydrochloride) or piperonyl butoxide (a pesticide synergist) prevents their hepatotoxicity (Reid et al., 1973; Reid and Krishna, 1973).

Just as there are species difference in metabolism there are also differences in the rate and degree of metabolism of a halogenated benzene in different organs, i.e., lung may differ from kidney which differs from liver, etc. Phenobarbital does not increase covalent binding in the lung as shown for the liver if administered before exposure to o-DCB (Reid, et al., 1973; see Table VII-3). Nevertheless, both the lung and kidney exhibit pathology following exposure to the dichlorobenzenes (Hollingsworth, et al., 1956; Battelle-Columbus, 1979b). The primary mechanism of toxicity in these tissues is likely to be the same as that described in the liver: necrosis due to binding of the reactive metabolite to cellular and tissue proteins, thereby interfering with the normal physiological and biochemical processes.

The halobenzenes, the dichlorobenzenes among them, also have been shown to induce porphyria (Rimington and Ziegler, 1963; Carlson, 1977, Battelle-Columbus, 1978c, 1978i, 1979a, 1979b). This condition, a disturbance in porphyrin metabolism, is characterized by increased formation and excretion of porphyrin precursors,

Table VII-2

Effect of Phenobarbital and SKF-525A Administration on Covalent Binding of Halogenated Benzene Derivatives to Rat Liver Protein In Vivo 6 Hours After Administration

(Reid et al., 1973; Reid and Krishna, 1973)
(After Ware and West, 1977)

	Control (nM/mg protein + S.E.) (N=6)	Phenobarbital (nM/mg protein + S.E.) (N=6)	Phenobarbital + SKF-525A ^a (nM/mg protein + S.E.) (N=6)
Monobromobenzene- ¹⁴ C (1 mM/kg)	0.267 ± 0.034	0.550 ± 0.031 ⁺	0.036 ± 0.024 [†]
Monochlorobenzene- ¹⁴ C (1 mM/kg)	0.364 ± 0.053	1.268 ± 0.278 ⁺	0.438 ± 0.144 ^{\$}
Moniodobenzene- ¹⁴ C (1 mM/kg)	0.090 ± 0.015	0.545 ± 0.129 ⁺	0.666 ± 0.304
Monofluorobenzene- ¹⁴ C (1 mM/kg)	0.085 ± 0.015	0.054 ± 0.008	0.052 ± 0.005
o-Dichlorobenzene- ¹⁴ C (0.5 mM/kg)	0.234 ± 0.015	0.308 ± 0.038	0.186 ± 0.014 ^{\$}
p-Dichlorobenzene- ¹⁴ C (0.5 mM/kg)	0.021 ± 0.002	0.012 ± 0.001 ⁺	0.006 ± 0.001 [†]

^a Diethylaminoethyl-2,2-diphenylvalerate hydrochloride (SKF-525A) (75 mg/kg i.p.) was given 1 hour before the hepatotoxin.

⁺ p < 0.01 compared with controls.

[†] p < 0.01 compared with phenobarbital alone.

^{\$} p < 0.02 compared with phenobarbital alone.

Table VII-3

Binding of Aromatic Hydrocarbons in Rat Lung:
Effect of Phenobarbital*

(Reid et al., 1973)
(Modified from Ware and West, 1977)

Compound	Time of Sacrifice (hour)	Binding of Hydrocarbon in Lung (nmole/mg protein)	
		Control	Phenobarbital
o-Dichlorobenzene- ¹⁴ C, 0.5 mM/kg	6	27.3 ± 1.4	18.9 ± 2.7 (p<0.05)
	24	20.9 ± 2.4	15.3 ± 2.2
p-Dichlorobenzene- ¹⁴ C, 0.5 mM/kg	6	4.6 ± 0.2	3.4 ± 0.5
	24	3.4 ± 0.4	1.8 ± 0.2

* Values are the means ± SE of 6 animals.

cutaneous photosensitivity, frequent hemolytic anemia and splenomegaly. Acute abdominal and nervous system manifestations may also occur. As was described in some detail earlier, exposure to the dichlorobenzenes leads to the induction of the mitochondrial enzyme, Δ -aminolevulinic acid synthetase, resulting in an increased production from heme of aminolevulinic acid and then other constituents of the heme synthetic pathway (Rimington and Ziegler, 1963; Poland, et al., 1971; Ariyoshi, et al., 1975). When these levels exceed those needed for maintenance of the system, clinical symptoms may be manifested.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Adjusted Acceptable Daily Intake (ADI) for the chemical is determined. For ingestion data, this approach is illustrated as follows:

$$\text{Adjusted ADI} = \frac{(\text{NOAEL or MEL in mg/kg})(70 \text{ kg})}{(\text{Uncertainty factor})(2 \text{ liters/day})}$$

The 70 kg adult consuming 2 liters of water per day is used as the basis for the calculations. A "no-observed-adverse-effect-level" or a "minimal-effect-level" is determined from animal toxicity data or human effects data. This level is divided by an uncertainty factor because, for these numbers which are derived from animal studies, there is no universally acceptable quantitative method to extrapolate from animals to humans, and the possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human toxicity data, an uncertainty factor is used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxins. The guidelines set forth by the National Academy of Sciences (Drinking Water and Health, Vol. 1, 1977) are used in establishing uncertainty factors. These guidelines are as follows: an uncertainty factor of 10 is used if there exist valid experimental results on ingestion by humans, an uncertainty factor of 100 is used if there exist valid results on long-

term feeding studies on experimental animals, and an uncertainty factor of 1000 is used if only limited data are available.

In the quantification of carcinogenic effects, mathematical models are used to calculate the estimated excess cancer risks associated with the consumption of a chemical through the drinking water. EPA's Carcinogen Assessment Group has used the multistage model, which is linear at low doses and does not exhibit a threshold, to extrapolate from high dose animal studies to low doses of the chemical expected in the environment. This model estimates the upper bound (95% confidence limit) of the incremental excess cancer rate that would be projected at a specific exposure level for a 70 kg adult, consuming 2 liters of water per day, over a 70 year lifespan. Excess cancer risk rates also can be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. Current understanding of the biological mechanisms involved in cancer do not allow for choosing among the models. The estimates of incremental risks associated with exposure to low doses of potential carcinogens can differ by several orders of magnitude when these models are applied. The linear, non-threshold multi-stage model often gives one of the highest risk estimates per dose and thus would usually be the one most consistent with a regulatory philosophy which would avoid underestimating potential risk.

The scientific data base, which is used to support the estimating of risk rate levels as well as other scientific

endeavors, has an inherent uncertainty. In addition, in many areas, there exists only limited knowledge concerning the health effects of contaminants at levels found in drinking water. Thus, the dose-response data gathered at high levels of exposure are used for extrapolation to estimate responses at levels of exposure nearer to the range in which a standard might be set. In most cases, data exist only for animals; thus, uncertainty exists when the data are extrapolated to humans. When estimating risk rate levels, several other areas of uncertainty exist such as the effect of age, sex, species and target organ of the test animals used in the experiment, as well as the exposure mode and dosing rates. Additional uncertainty exists when there is exposure to more than one contaminant due to the lack of information about possible additive, synergistic or antagonistic interactions.

Non-Carcinogenic Effects

The principal toxic effects of the dichlorobenzenes in humans and other animals from both acute and longer-term exposures include central nervous system (CNS) depression, blood dyscrasias (granulocytopenia, hemolytic anemia and leukemias), lung, kidney and liver damage. In addition to liver necrosis, the dichlorobenzenes also can produce porphyria. The appearance and intensity of these and other adverse effects are dependent upon dose and duration of exposure. Death following high level acute exposure usually results from the CNS effects (primarily, respiratory failure). Deaths in humans have been reported following accidental exposure.

Several investigators have determined the acute lethal dose levels after exposure to ortho- and para-dichlorobenzene in several species. These data are summarized in Table VIII-1 (same as Table V-3).

Varshavskaya (1968), in her comparative studies on the adverse effects of the lower chlorinated benzenes, showed that, when determining the LD₅₀s, o-DCB was slightly less toxic in mice and rats than was monochlorobenzene (MCB), and that p-DCB was even less toxic than either MCB or o-DCB. o-DCB was slightly more toxic than MCB and p-DCB in rabbits and guinea pigs. Thus, in general, one may conclude that o-DCB is acutely more toxic than is p-DCB.

A number of studies with o-dichlorobenzene and p-dichlorobenzene are available in which dose-response data are described and which allow the identification of no-observed-adverse-effect-levels (NOAELs) following longer-term or lifetime periods of exposure. Animals were exposed both by gavage and by inhalation for periods of time constituting a subchronic or chronic exposure. Since several gavage studies are available for evaluation, only these, and not the inhalation studies, will be used in the quantification of toxicological effects, as this route of exposure is more appropriate for the development of allowable exposure levels in drinking water. Comparable studies with the meta- isomer of dichlorobenzene have not been reported. Therefore, it will be assumed that ADIs developed for o-dichlorobenzene also will be appropriate for m-dichlorobenzene. This assumption is defensible for several

Table VIII-1
Acute Toxicity Data for o- and p-Dichlorobenzene

<u>Animal</u>	<u>Route</u>	<u>LD50</u>	<u>LCL0</u>	<u>Reference</u>
<u>o-Dichlorobenzene</u>				
Rat	Oral	500 mg/kg		NIOSH, 1978
Rat	Oral	2138 mg/l		Varshavskaya, 1968
Mouse	Oral	2000 mg/l		Varshavskaya, 1968
Rabbit	Oral	1875 mg/l		Varshavskaya, 1968
Guinea Pig	Oral	3375 mg/l		Varshavskaya, 1968
Guinea Pig	Oral	2000 mg/kg		Hollingsworth, et al., 1958
Rat	Inhal.		821 ppm/7 hr	Hollingsworth, 1958
Guinea Pig	Inhal.		800 ppm/7 hr	Hollingsworth, 1958
Guinea Pig	Inhal.		800 ppm/24 hr	Cameron, et al., 1937
<u>p-Dichlorobenzene</u>				
Rat	Oral	500 mg/kg		NIOSH, 1978
Rat	Oral	2500 mg/kg		Hollingsworth, et al., 1956
Rat	Oral	2138 mg/l		Varshavskaya, 1968
Mouse	Oral	3220 mg/l		Varshavskaya, 1968
Rabbit	Oral	2812 mg/l		Varshavskaya, 1968
Guinea pig	Oral	7593 mg/l		Varshavskaya, 1968
Guinea pig	Oral	2800 mg/kg		Hollingsworth, et al., 1956
Mouse	SC	5145 mg/kg	(LDL0)	Irie, et al., 1973

reasons: 1) in general, in mutagenicity and other short-term tests, the meta isomer behaved more like the ortho isomer than like the para isomer, and 2) short- and longer-term studies with o- and p-DCB suggest that the ortho isomer is somewhat more toxic than the para isomer on a mg/kg basis. Thus, to assume that the meta isomer is more similar to the ortho isomer would be consistent with a regulatory philosophy that seeks to avoid underestimating the potential risk to human health.

o-Dichlorobenzene (and/or m-Dichlorobenzene)

Hollingsworth, et al. (1958) gave rats a series of 138 doses of o-DCB over a period of 192 days (18.8, 188 or 376 mg/kg/day, five days a week) by intubation. No adverse effects were observed at the lowest dose. With the intermediate dose, a slight increase in liver and kidney weight was noted. At the highest dose, there was a slight decrease in the weight of the spleen and a modest increase in the weight of the liver accompanied by cloudy swelling.

If one were to assume that the results of this study were appropriate for use in developing an acceptable daily intake (ADI), it would be derived thusly:

$$\frac{18.8 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times \frac{5}{7}}{100 \times 10} = 0.94 \text{ mg/day}$$

(for a 70 kg adult)

Where: 18.8 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Varshavskaya (1968) administered o-DCB orally to white rats for nine months at doses of 0.001, 0.01 or 0.1 mg/kg/day. Effects were observed at the two higher doses. The author reported an inhibition of mitosis in the bone marrow, as well as neutropenia and abnormal conditioned reflexes. These changes in the blood profile can be important in that they could be precursors to pancytopenia or leukemia. In this study, however, no carcinogenic activity was observed. Also, at the two higher doses, there was an increase in acid phosphatase and a decrease in alkaline phosphatase. At the highest dose, a marked increase in the amount of 17-ketosteroids in the urine occurred. This was attributed to hyperplasia of the adrenal cortex, as an increase in adrenal weight and a decrease in ascorbic acid content of the adrenals also were observed. The 0.001 mg/kg/day dose had no observable effects on any of the parameters studied.

If one were to assume that the results of the Varshavskaya study were appropriate for use in developing an ADI for o-DCB, it could be derived thusly:

$$\frac{0.001 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0}{100 \times 10} = 0.00007 \text{ mg/day}$$

(for a 70 kg adult)

Where: 0.001 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Subchronic gavage studies with o-DCB in mice and rats were conducted to assist in dosage selection for the NTP 104-week carcinogenicity study (Battelle-Columbus, 1978c,i). Single doses in corn oil were administered 5 days/week for 13 weeks. Treated mice received doses of 30, 60, 125, 250 or 500 mg/kg/day; treated rats received 30, 60, 125, 250 or 500 mg/kg/day. Controls received corn oil. Other protocol details are described in Chapter V.

In the mice, body weight gain was decreased significantly in animals of both sexes at the 500 mg/kg dose level and in males at the 250 mg/kg dose. Of the hematological parameters tested, white cell counts of treated males were lower than those of control males. It was suggested that this was due to lower

than normal control values, as observed typically in that laboratory. Since individual data were not available for statistical analysis, it cannot be shown whether or not the differences between the controls and the treated groups were statistically significant. But, since there is at least anecdotal evidence to suggest a possible relationship between exposure to o-DCB and leukemia in humans, it would be prudent to evaluate these results in greater depth.

Increased, but apparently not statistically-significant, blood alkaline phosphatase levels were observed in males receiving 125 and 250 mg o-DCB/kg/day. SGPT levels were increased significantly in the two surviving males receiving 500 mg/kg/day, due to the high value for the one animal exhibiting hepatocellular necrosis. Urine volume was greater in the treated animals than in controls, but no record of fluid intake was kept. The significance of this observation is unknown, since no parameters of the urinalysis measured were altered, except for the decreases in specific gravity and creatinine levels.

Males receiving 500 mg/kg exhibited higher uroporphyrin levels than did male controls; females at that dose showed higher coproporphyrin levels. These parameters were not measured in the other dose groups. In addition, a dose-dependent increase in liver protoporphyrin was observed in the females, but not in the males. Even without statistical evaluation, one could observe that liver weights in the highest dose group of both sexes were increased significantly. An increase of lesser magnitude

was observed in the females at the 250 mg/kg/day dose level. Histopathological examination of several tissues revealed that no lesions were apparent in animals treated with 125 mg/kg/day or lower doses of o-DCB.

Interpretation of the results of the study suggests that an oral NOAEL of 125 mg/kg/day could be identified in mice, if one discounts the observations concerning the white cell counts. In the absence of the raw data, at this time, it will be assumed that the investigators have interpreted this finding correctly. Under these circumstances, if one were to assume that the results of the mouse 90-day study were appropriate for use in developing an ADI, it could be derived thusly:

$$\frac{125 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 6.25 \text{ mg/day}$$

(for a 70 kg adult)

Where: 125 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration of significantly less than lifetime

In the rats, body weight gain was decreased significantly at the two higher doses (250 and 500 mg/kg/day). Cholesterol levels were increased in males at the two higher doses and in females at the three higher doses. The combined alpha-globulin

fraction appeared to be increased in females at the highest dose and males at the two highest doses. As in the mice, urinary output was increased substantially, with concomitant decreases in specific gravity and creatinine.

Both uro- and coproporphyrin levels in the urine increased significantly in animals receiving 500 mg/kg/day. No measurements of these parameters were made in other dose groups. However, liver protoporphyrin levels were not changed. Absolute and relative liver weights were increased in the 250 and 500 mg/kg/day groups. Histopathological examination of tissues revealed liver and kidney changes in the highest dose group, and liver changes in the 250 mg/kg/day groups. As for the mice, 125 mg/kg/day was identified as the NOAEL for the rats.

If one were to assume that the results of the study in which rats were exposed to o-DCB subchronically were appropriate for use in developing an ADI, it could be derived thusly:

$$\frac{125 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 6.25 \text{ mg/day}$$

(for a 70 kg adult)

Where: 125 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing
regimen to 7 day/week

100 = uncertainty factor, appropriate for
use with NOAEL from animal studies
with no comparable human data
(longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Chronic (104-week) gavage studies with o-DCB in mice and rats were conducted in the NTP carcinogenicity study (NTP, 1982). The results were presented in a preliminary report not yet approved by the NTP Board of Scientific Counselors. Single daily doses of o-DCB in corn oil were administered 5 days/week. Treated animals of both species received doses of 60 or 120 mg/kg/day. Controls received corn oil alone. Other protocol details are described in Chapter V.

In the mice, no differences in survival rates were observed between treated and control groups of either sex. There also was no evidence of any compound-related non-neoplastic liver lesions, suggesting that the maximum tolerated dose was not administered in this study and that a higher dose could have been tolerated.

If the results of the mouse chronic study were considered appropriate for use in developing an ADI, it could be derived thusly:

$$\frac{120 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 7} = 60 \text{ mg/day}$$

(for a 70 kg adult)

Where: 120 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (lifetime exposure)

In the rats during the NTP bioassay, there was no significant difference in the survival rates of female rats at either dose or of males at the low dose when compared with controls. However, there was a statistically significant decrease in survival in the high dose males ($P < 0.001$). This difference appeared to be due, primarily, to gavage error. As with the mice, there were no non-neoplastic lesions of the liver in rats of either sex receiving either dose of o-DCB. Again, this suggests that the maximum tolerated dose was not achieved and a higher dose should have been administered.

If the results of the rat chronic study were considered appropriate for use in developing an ADI, it could be derived thusly:

$$\frac{120 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 7} = 60 \text{ mg/day}$$

(for a 70 kg adult)

Where: 120 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (lifetime exposure)

p-Dichlorobenzene

Hollingsworth, et al. (1956) administered 138 oral doses of p-DCB at 18.8, 188 or 376 mg/kg/day to rats over a 192-day period (5 days/week). At the highest dose (376 mg/kg/day), there was an increase in the weights of the spleen, kidney and liver accompanied by slight cirrhosis and focal necrosis in the liver. There was also cloudy swelling of the renal tubular epithelium. At the intermediate dose (188 mg/kg/day), liver and kidney weights were increased. No adverse effects were noted at the lowest dose (18.8 mg/kg/day).

If one were assume that the results of this study were appropriate for use in developing an acceptable daily intake (ADI), it would be derived thusly:

$$\frac{18.8 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 0.94 \text{ mg/day}$$

(for a 70 kg adult)

Where: 18.8 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Subchronic gavage studies with p-DCB in mice and rats were conducted to assist in dosage selection for the NTP 104-week carcinogenicity study (Battelle-Columbus, 1979a,b;1980a,b). The 1979 studies had to be repeated because no no-effect level could be identified in mice receiving 600, 900, 1000, 1500 or 1800 mg/kg/day or in rats receiving 300, 600, 900, 1200 or 1500 mg/kg/day. In the repeat subchronic studies, the animals received single doses of p-DCB in corn oil 5 days/week for 13 weeks. Treated mice were administered doses of 84.4, 168.8, 337.5, 675 or 800 mg p-DCB/kg/day; rats were administered 37.5, 75, 150, 300 or 600 mg p-DCB/kg/day. Controls received corn oil. Other protocol details are described in Chapter V.

In the mice, no significant differences were apparent in dietary consumption or relative body weight gains between treated and control animals of either sex. Upon histopathological examination, it was observed that animals in the two highest dose groups exhibited a significant degree of centrilobular to midzonal hepatocytomegaly. Few changes of this nature were observed in animals receiving 337.5 mg/kg/day. This dose was determined to be the NOAEL.

If the results of this study in mice were considered appropriate for use in the developing of an ADI, it would derived thusly:

$$\frac{337.5 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 16.9 \text{ mg/day}$$

(for a 70 kg adult)

Where: 337.5 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

In the rats, no significant differences in food consumption or body weight gain were observed between treated and control animals of either sex at any dose. Microscopically, there was an increased incidence and severity of renal cortical degeneration in males receiving the two highest doses, as had been observed in the first subchronic rat study. Females showed no significant changes at any dose level. The NOAEL for this study was established at 150 mg/kg/day.

If the results of this rat subchronic study were considered to be appropriate for use in developing an ADI, it could be derived thusly:

$$\frac{150 \text{ mg/kg/day} \times 70 \text{ kg} \times 1.0 \times 5}{100 \times 10 \times 7} = 7.5 \text{ mg/day}$$

(for a 70 kg adult)

Where: 150 mg/kg/day = NOAEL

70 kg = weight of protected individual

1.0 = ratio of administered dose absorbed

5/7 = conversion of 5 day/week dosing regimen to 7 day/week

100 = uncertainty factor, appropriate for use with NOAEL from animal studies with no comparable human data (longer-term exposure duration)

10 = uncertainty factor, appropriate for use with data from exposure duration significantly less than lifetime

Quantification of Non-carcinogenic Effects

. Table VIII-2 summarizes the ADIs derived from the available gavage studies on o- and p-dichlorobenzene which contain adequate dose-response data identifying NOAELs. As can be seen, a wide range of numbers was derived. For o-DCB (and m-DCB), the ADIs range from 0.00007 mg/day (Varshavskaya, 1968) to 60 mg/day (NTP, 1982; preliminary report). For p-DCB, the ADIs range from 0.94 mg/day (Hollingsworth, *et al.*, 1956) to 16.9 mg/day (Battelle-Columbus, 1980a). However, a rationale can be presented by which certain of these numbers can be eliminated and others supported.

Ortho-dichlorobenzene (and, meta-dichlorobenzene)

While the Varshavskaya study suggests that effects can be seen at very low doses when compared with the other studies, little in the way of quantitative experimental detail was presented in her publication. Therefore, it is difficult to

Table VIII-2

Possible ADIs for the Dichlorobenzenes

Compound	Experiment	Possible ADI
o-DCB/m-DCB	Hollingsworth, et al. (1958) subchronic rat	0.94 mg/day
	Varshavskaya (1968) subchronic rat	0.07 ug/day
	Battelle-Columbus (1978c) subchronic mouse	6.25 mg/day
	Battelle-Columbus (1978i) subchronic rat	6.25 mg/day
	NTP (1982) chronic mouse (preliminary report)	60 mg/day
	NTP (1982) chronic rat (preliminary report)	60 mg/day

p-DCB	Hollingsworth, <u>et al.</u> (1956) subchronic rat	0.94 mg/day
	Battelle-Columbus (1980a) subchronic mouse	16.9 mg/day
	Battelle-Columbus (1980b) subchronic rat	7.5 mg/day

assess fully the results presented, and one cannot conclude that this paper should be the basis for the development of an Adjusted Acceptable Daily Intake (AADI).

The fact that the ADIs generated from the chronic studies in the NTP bioassay are larger than those derived from the subchronic studies preceding them would suggest that the 10-fold uncertainty factor used to estimate a chronic ADI from subchronic data may be unnecessarily large for this compound. However, the NTP Board of Scientific Counselors has not yet approved the report prepared on the bioassay of o-DCB. Therefore, it is prudent to reserve judgment on its validity until such time as the report is approved.

The results of Hollingsworth, et al. (1958) suggest an ADI of 0.94 mg/day while those of the subchronic studies preceding the NTP bioassay suggest an ADI of 6.25 mg/day. Each ADI was derived from an NOAEL (18.8 mg/kg vs. 125 mg/kg, respectively). Since the highest NOAEL should be used to derive an ADI, it is more appropriate to use the NOAEL established in the NTP subchronic studies, than the NOAEL from the Hollingsworth study. In addition, the NTP subchronic studies employed additional doses (5 vs 3), thereby allowing for a more precise identification of a NOAEL. It also should be noted that the minimal effect dose identified in the Hollingsworth study (188.8 mg/kg) is somewhat higher than the NOAEL established in the NTP subchronic studies.

For o-DCB (and, m-DCB), then, if one were to use the ADI from the NTP subchronic studies to determine the AADI, it would be derived thusly:

$$\text{AADI} = \frac{\text{ADI}}{21} = \frac{6.25 \text{ mg/day}}{21} = 3.125 \text{ mg/l/day}$$

This AADI assumes that the protected individual (a 70 kg adult) drinks 2 liters of water/day and that the sole source of exposure to o- or m-dichlorobenzene is via that drinking water. [It is important to note that the odor threshold for o-DCB and m-DCB in water has been identified as 0.01 and 0.02 ppm, respectively (Kolle, 1972). Therefore, any MCL for these compounds may have to consider the aesthetic, as well as the toxic, consequences of exposure to these compounds in drinking water].

p-Dichlorobenzene

The results of the Hollingsworth, et al. (1956) study suggest an ADI of 0.94 mg/day while those from the subchronic studies preceding the NTP bioassay suggest ADIs of 7.5 mg/day (rats) and 16.9 mg/day (mice). It is apparent from these three studies, as well as the acute toxicity studies described earlier, that the rat is more sensitive to p-DCB toxicity than is the mouse. Therefore, to be consistent with the philosophy that one uses data from the most sensitive animal species when estimating the potential risk to the human, the data from the experiments in the rat should be used in deriving an AADI.

The ADI derived from the Hollingsworth study was based upon an NOAEL of 18.8 mg/kg; the ADI from the NTP subchronic study in the rat was derived from an NOAEL of 150 mg/kg.

Since the highest NOAEL should be used to derive an ADI, it is more appropriate to use the NOAEL established in the NTP subchronic study than the NOAEL from the Hollingsworth study. The NTP subchronic study employed additional treatment doses (5 vs. 3), thereby allowing for a more precise identification of an NOAEL. In addition, it should be noted that the minimal effect level identified in the Hollingsworth study (188 mg/kg) was somewhat higher than the NOAEL established in the NTP subchronic study.

As with o-DCB (and m-DCB), it may be that any ADIs derived from the as yet unreported NTP chronic studies would be larger than those derived from the subchronic studies preceding them because the 10-fold uncertainty factor applied to accommodate for the difference in duration of exposure may be unnecessarily large. However, until the report has been approved by the NTP Board of Scientific Counselors and published as final, it is appropriate to develop an ADI and AADI based upon the subchronic data. The AADI can be reevaluated at a later date.

For p-DCB, then, if one were to use the ADI from the NTP subchronic study in rats to determine the AADI, it would be derived thusly:

$$\text{AADI} = \frac{\text{ADI}}{21} = \frac{7.5 \text{ mg/day}}{21} = 3.75 \text{ mg/l/day}$$

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